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Water Quality and Pollutant Source Monitoring Field and Laboratory Procedures. Training Manual

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Technology Center, Cincinnati, OH

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Water

EPA

Water Quality & Pollutant Source Monitoring: Field and Laboratory Procedures

Training Manual

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This report is designed for personnel engaged in programs concerned with monitoring the quality of surface waters. The report describes suitable methods, techniques, and instrumentation used in field and laboratory sampling and measurements in the chemical, biological, and bacteriological areas of water quality monitoring.

KEYWORDS: *Monitoring, *Water pollution.

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Water Quality & Pollutant Source Monitoring: Field and Laboratory Procedures

report

This course is designed for personnel engaged in programs concerned with monitoring the quality of surface waters. Interdisciplinary needs and responsibilities in the accumulation and interpretation of data from field and laboratory activities are emphasized.

The report describes

Upon completion of the course the student will be able to apply suitable methods, techniques, and instrumentation used in field sampling and measurements, and will be familiar with basic laboratory techniques and procedures in the chemical, biological, and bacteriological areas. Advantages and limitations of equipment, methods and techniques will be considered.

of water quality monitoring

and laboratory

U. S. ENVIRONMENTAL PROTECTION AGENCY
Office of Water Program Operations
National Training and Operational Technology Center

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Reference to commercial products, trade names, or manufacturers is for purposes of example and illustration. Such references do not constitute endorsement by the Office of Water Program Operations, U. S. Environmental Protection Agency.

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FLOW MEASUREMENT

I INTRODUCTION

A Flow measurements are among the more important data collected during a water quality survey. Such measurements are used to interpret data variations, calculate loadings, and expedite survey planning. If the analysis of survey data involves estimation of loads, the accurate measurement of discharge assumes a level of importance equal to that of laboratory and analytical results.

In the following discussion, procedures for measurement of stream flow and waste discharge are described. Some of these procedures are used in long-term, very detailed water quality and supply studies; others are more suited to short-term pollution surveys.

B In accordance with EPA policy, units in this outline are expressed in the metric system. The equivalent English system units appear in parentheses following the metric designation. Tables extracted from the literature have been left in the form in which they were originally given. Applicable conversion factors appear in Section IV of this outline.

II PLANNING

A Station Location

Four factors influence location of gauging or flow measurement stations:

1 Survey objectives

2 Physical accessibility

3 Characteristics of the stream bed

4 Hydrologic effects

Survey objectives represent the major influence on station location; depending upon objectives, gauging stations may be located above and/or below confluences and outfalls.

Physical accessibility determines the ease and cost of installation and maintenance of the station. The characteristics of the stream bed may greatly influence the obtainable accuracy of measurement. For instance, rocky bottoms greatly reduce the accuracy of current meters. Sedimentation in pools behind control structures may influence stage-discharge relationships. Hydrologic variations in stream flow may cause washout or bypass of the gauging station. In the Southwest, flash floods have been known to wash out or bypass gauging stations by assuming different channels of flow.

B Methodology

Choice of a specific measurement procedure is dependent upon at least three considerations:

1 The relation between obtainable and desired accuracy

2 Overall cost of measurement

3 The quantity of flow to be measured

Ideally, discharge measurements should be reported to a specific degree of accuracy: the gauging procedure greatly influences this accuracy. The influence of overall cost on the gauging program is readily apparent. Extensive, detailed studies are usually characterized by high costs for automatic instrumentation and low personnel cost; the opposite is usually true for less detailed studies. The range of flows to be measured (within acceptable accuracy) is, of course, not known prior to the survey. However, experienced personnel usually can make reasonable estimates of expected flows from visual observations and other data, and may recommend appropriate gauging procedures. In this regard, experienced personnel always should be consulted.

III MEASUREMENT

A Streams, Rivers, and Open Channels

1 Current Meter

The current meter is a device for measuring the velocity of a flowing body of water. The stream cross section is divided into a number of smaller sections, and the average velocity in each section is determined. The discharge is then found by summing the products of area and velocity of each section.

2 Stage-discharge relationships

Large flows usually are measured by development of and reference to a stage-discharge curve; this procedure has long been used by the U. S. Geological Survey Such gauging stations are composed of a control structure located downstream of the location of measurement and some type of water level indicator which identifies the height of the water surface above a previously determined datum.

3 Weirs

A weir may be defined as a dam or impediment to flow, over which the discharge conforms to an equation. The edge or top surface over which the liquid flows is called the weir crest. The sheet of liquid falling over the weir is called the nappe. The difference in elevation between the crest and the liquid surface at a specified location, usually a point upstream, is called the weir head. Head-discharge equations based on precise installation requirements have been developed for each type of weir. Weirs so installed are called standard weirs. Equations for non-standard installations or unusual types may be derived empirically.

Weirs are simple, reliable measurement devices and have been investigated extensively in controlled experiments. They are usually installed to obtain continuous or semi-continuous records of discharge. Limitations of weirs include difficulty during installation, potential siltation in the weir pond, and a relatively high head requirement, 0.12 - 0.61 m (0.4 - 2.0 feet). Frequent errors in weir installation include insufficient attention to standard installation requirements and failure to assure completely free discharge of the nappe.

a Standard suppressed rectangular weir

This type of weir is essentially a dam placed across a channel. The height of the crest is so controlled that construction of the nappe in the vertical direction is fully developed. Since the ends of the weir are coincident with the sides of the channel lateral contraction is impossible. This weir requires a channel of rectangular cross section, other special installation conditions, and is rarely used in plant survey work. It is more commonly used to measure the discharge of small streams.

The standard equation for discharge of a suppressed rectangular weir (Francis equation) is:

$$Q = 3.33\ LH^{3/2}$$

where

Q = discharge, m^3/sec (cfs)
L = length of the weir crest, meters (feet)
H = weir head, meters (feet)

The performance of this type of weir has been experimentally investigated more intensively than that of other weirs. At least six forms of the discharge equation are commonly employed. The standard suppressed weir is sometimes used when data must be unusually reliable.

b Standard contracted rectangular weir

The crest of this type of weir is shaped like a rectangular notch The sides and level edge of the crest are so removed from the sides and bottom of the channel that contraction of the nappe is fully developed in all directions. This weir is commonly used in both plant surveys and measurement of stream discharge

The standard equation for discharge of a contracted rectangular weir (corrected Francis equation) is

$$Q = 3.33\ (L - 0.2H)H^{3/2}$$

where

Q = discharge, m^3/sec (cfs)
L = length of the level crest edge, meters (feet)
H = weir head, meters (feet)
$0.2H$ = correction for end contractions as proposed by Francis

c Cipolletti weir

The Cipolletti weir is similar to the contracted rectangular weir except that the sides of the weir notch are inclined outward at a slope of 1 horizontal to 4 vertical. Discharge through a Cipolletti weir occurs as though end contractions were absent and the standard equation does not include a corresponding factor for correction.

RECTANGULAR WEIR

The standard equation for discharge through a Cipolletti weir is

$$Q = 3.367\ LH^{3/2}$$

where

Q = discharge, m^3/sec (cfs)

L = length of the level crest edge, meters (feet)

H = weir head, meters (feet)

The discharge of a Cipolletti weir exceeds that of a suppressed rectangular weir of equal crest length by approximately 1 percent.

d Triangular weirs

The crest of a triangular weir is shaped like a V-notch with sides equally inclined from the vertical. The central angle of the notch is normally 60 or 90 degrees. Since the triangular weir develops more head at a given discharge than does a rectangular shape, it is especially useful for measurement of small or varying flow. It is preferred for discharges less than 28 L/sec (1 cfs), is as accurate as other shapes up to 280 L/sec (10 cfs), and is commonly used in plant surveys.

The standard equation for discharge of a 90° triangular weir (Cone formula) is

$$Q = 2.49H^{2.48}$$

where

Q = discharge, m^3/sec (cfs)

H = weir head, meters (feet)

Crest height and head are measured to and from the point of the notch, respectively.

e Accuracy and installation requirements

Quotations of weir accuracy express the difference in performance between two purportedly identical weirs and do not include the effects of random error in measurement of head. Weirs installed according to the following specifications should measure discharge within ± 5% of the values observed when the previously cited standard equations were developed.

1) The upstream face of the bulkhead and/or weir plate shall be smooth and in a vertical plane perpendicular to the axis of the channel.

2) The crest edge shall be level, shall have a square upstream corner, and shall not exceed 2 mm (0.08 in) in thickness. If the weir plate is thicker than the prescribed crest thickness the downstream corner of the crest shall be relieved by a 45° chamfer.

3) The pressure under the nappe shall be atmospheric. The maximum water surface in the downstream channel shall be at least 60 mm (0.2 ft.) below the weir crest. Vents shall be provided at the ends of standard suppressed weirs to admit air to the space beneath the nappe.

4) The approach channel shall be straight and of uniform cross section for a distance above the weir of 15 to 20 times the maximum head, or shall be so baffled that a normal distribution of velocities exists in the flow approaching the crest and the water surface at the point of head measurement is free of disturbances. The cross-sectional area of the approach channel shall be at least 6 times the maximum area of the nappe at the crest.

5) The height of the crest above the bottom of the approach channel shall be at least twice, and preferably 3 times, the maximum head and not less than, 0.3 m (1 foot). For the standard suppressed weir the crest height shall be 5 times the maximum head. The height of triangular weirs shall be measured from the channel bottom to the point of the notch.

6) There shall be a clearance of at least 3 times the maximum head between the sides of the channel and the intersection of the maximum water surface with the sides of the weir notch.

7) For standard rectangular suppressed, rectangular contracted, and Cipolletti weirs the maximum head shall not exceed 1/3 the length of the level crest edge.

8) The head on the weir shall be taken as the difference in elevation between the crest and the water surface at a point upstream a distance of 4 to 10 times the maximum head or a minimum of 1.8 m (6 feet).

9) The head used to compute discharge shall be the mean of at least 10 separate measurements taken at equal intervals. The head range of the measuring device shall be 6 - 46 cm (0.2 - 1.5 feet).

The capacities of weirs which conform to these specifications are indicated in Table 1.

4 Parshall flume

The Parshall flume is an open constricted channel in which the rate of flow is related to the upstream head or to the difference between upstream and downstream heads. It consists of an entrance section with converging vertical walls and level floor, a throat section with parallel walls and floor declining downstream, and an exit section with diverging walls and floor inclining downstream. Plan and sectional views are shown in Figure 2. Advantages of the Parshall flume include a low head requirement, dependable accuracy, large capacity range, and self cleaning capability. Its primary disadvantage is the high cost of fabrication; this cost may be avoided by use of a prefabricated flume. Use of prefabricated flumes during plant surveys is becoming increasingly popular.

a Standard equations

The dimensions of Parshall flumes are specified to insure agreement with standard equations. Table of dimensions are available from several sources[3,4]. For flumes of 15 cm (6 inch) to 2.4 m (8 foot) throat width the following standard equations have been developed.

1) 15 cm (6 inch) throat width

$$Q = 2.06\ H_a^{1.58}$$

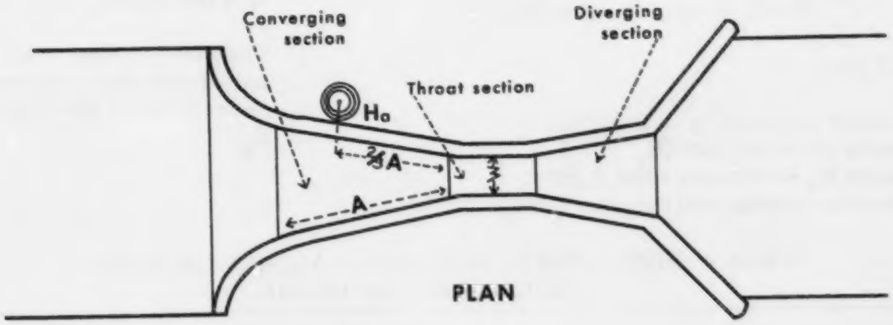
2) 23 cm (9 inch) throat width

$$Q = 3.07\ H_a^{1.53}$$

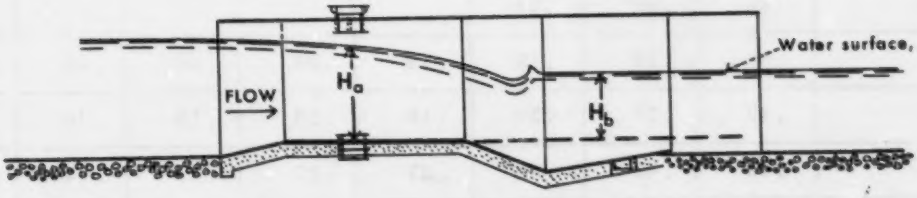
TABLE 1 DISCHARGE OF STANDARD WEIRS

Crest Length (Feet)	Contracted Rectangular* Weir (discharge-cfs)		Suppressed Rectangular* Weir (discharge-cfs)		Cipolletti* Weir (discharge-cfs)		90° Triangular* Weir (discharge-cfs)	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
1.0	.590	.286	.631	.298	.638	.301		
1.5	1.65	.435	1.77	.447	1.79	.452		
2.0	3.34	.584	3.65	.596	3.69	.602		
2.5	5.87	.732	6.30	.744	6.37	.753		
3.0	9.32	.881	10.0	.893	10.1	.903		
3.5	13.8	1.03	14.8	1.04	15.0	1.05		
4.0	19.1	1.18	20.4	1.19	20.6	1.20		
4.5	25.6	1.33	27.5	1.34	27.8	1.35		
							6.55	.046
5.0	28.8	1.48	30.6	1.49	30.9	1.51		
6.0	34.9	1.78	36.7	1.79	37.1	1.81		
7.0	41.0	2.07	42.8	2.08	43.3	2.11		
8.0	47.1	2.37	48.9	2.38	49.5	2.41		
9.0	53.2	2.67	55.0	2.68	55.7	2.71		
10.0	59.3	2.97	61.1	2.98	62.0	3.01		

* $H \geq 0.2$ ft, $H \leq 1.5$ ft, $H \leq 1/3\ L$


Converging section
Diverging section
Throat section
H_a
$\frac{2}{3}A$
A
W

PLAN


Water surface, s
H_a
FLOW
H_b

SECTION

FIGURE 2 PARSHALL FLUME

3) 0.3 - 2.4 m (1 to 8 foot) throat width

$$Q = 4WH_a^{1.522W^{0.026}}$$

where

Q = free-flow discharge, defined as that condition which exists when the elevation of the downstream water surface above the crest, H_b, does not exceed a prescribed percentage of the upstream depth above the crest, H_a. The prescribed percentage of submergence is 60 percent for 15 and 23 cm (6 and 9 inch) flumes and 70 percent for 0.3 - 2.4 m (1 to 8) foot flumes

W = throat width, meters (feet)

H_a = upstream head above the flume crest, meters (feet)

b Head loss

The head required by a Parshall flume is greater than $(H_a - H_b)$ because H_a is measured at a point in the converging section where the water surface has already begun to decline. Table 2 indicates the total head requirements of standard Parshall flumes. These losses should be added to the normal channel depth to determine the elevation of the water surface at the flume entrance. No head losses are indicated for discharge-throat width combinations for which H_a is less than 6 cm (0.2 ft.) or greater than 2/3 the sidewall depth in the converging section.

c Accuracy and installation requirements

A Parshall flume will measure discharge within ± 5% of the standard value if the following conditions are observed.

1) The dimensions of the flume shall conform to standard specifications.

2) The downstream head, H_b, shall not exceed the recommended percentage of the upstream head, H_a.

TABLE 2 HEAD LOSS IN STANDARD PARSHALL FLUMES UNDER FREE DISCHARGE

Discharge (cfs)	Head Loss, Feet, in Flume of Indicated Width							
	1 foot	2 feet	3 feet	4 feet	5 feet	6 feet	7 feet	8 feet
0.5	.08							
1.0	.14	.09	.06					
2.5	.26	.16	.12	.10	.08	.07	.06	.05
5.0	.42	.27	.20	.16	.13	.12	.10	.09
10.0	.70	.45	.34	.27	.22	.19	.17	.15
30.0			.70	.56	.47	.40	.35	.30
50.0					.68	.57	.49	.41

$H_a \geq 0.2$, $H_a \leq 2.0$

3) The upstream head shall be measured in a stilling well connected to the flume by a pipe approximately 3.8 cm ($1\frac{1}{2}$ inches) in diameter.

4) The flume shall be installed in a straight channel with the centerline of the flume parallel to the direction of flow.

5) The flume shall be so chosen, installed, or baffled that a normal distribution of velocities exists at the flume entrance.

5 Tracer materials

Techniques, materials, and instruments are presently being refined to permit accurate measurement of instantaneous or steady flow with several tracer materials. Measurements are made by one of two methods:

a Continuous addition of tracer

b Slug injection

With the first method, tracer is injected into a stream at a continuous and uniform rate; with the second a single dose of tracer material is added. Both methods depend on good transverse mixing and uniform dispersion throughout a stream. The concentration of tracer material is measured downstream from the point of addition. When continuous addition is employed, flow rates are calculated from the equation:

$$q \cdot C = (Q + q)\, c$$

in which q = rate of tracer addition to the stream at concentration, C, Q = the stream flow rate, and c = the resulting concentration of the stream flow combined with the tracer. For the slug injection method

$$Q = \frac{S}{\bar{c}\ \Delta t}$$

in which Q = the stream discharge, S = the quantity of tracer added, $\bar{c}$ = the weighted average concentration of tracer material during its passage past the sampling point, and Δt = the total time of the sampling period. Disadvantages of tracer methods include incomplete mixing, natural adsorption and interference, and high equipment costs.

6 Floats

Floats may be used to estimate the time of travel between two points a known distance apart. The velocity so obtained may be multiplied by 0.85 to give the average velocity in the vertical. Knowing the mean velocity and the area of the flowing stream, the discharge may be estimated. Floats should be employed only when other methods are impractical.

B Pipes and Conduits

1 Weirs and Parshall flumes

Weirs and Parshall flumes are commonly installed in manholes and junction boxes and at outfalls to measure flow in pipes. All conditions required for measurement of open channel flow must be observed.

2 Tracer materials

These methods are popular for measurement of pipe flow because they do not require installation of equipment or modification of the flow. These are especially convenient for measurement of exfiltration and infiltration.

3 Depth-slope

If the depth of the flowing stream and the slope of the sewer invert are known, the discharge may be computed by means of any one of several formulas.

a Manning formula

$$Q = \frac{1.486}{n}\ A\, R^{2/3} S^{1/2}$$

where

Q = discharge, m^3/sec (cfs)

n = roughness coefficient

A = area of flow, m^2 (sq. ft.)

R = hydraulic radius

= area divided by wetted perimeter, m (ft).

S = slope

b Chezy formula

$$Q = CA\sqrt{RS}$$

where

Q = discharge, m^3/sec (cfs)

C = friction coefficient

A = area of flow, m^2(sq. ft.)

R = hydraulic radius, m (ft.)

= area divided by wetted perimeter

S = slope

C Head Measuring Devices

Several of the above gauging methods require the measurement of water level in order that discharge may be determined. Any device used for this purpose must be referenced to some zero elevation. For example, the zero elevation for weir measurements is the elevation of the weir crest. The choice of method is dependent upon the degree of accuracy and the type of record desired.

1 Hook gauge

The hook gauge measures water elevation from a fixed point. The hook is dropped below the water surface and then raised until the point of the hook just breaks the surface. This method probably will give the most precise results when properly applied.

2 Staff gauge

The staff gauge is merely a graduated scale placed in the water so that elevation may be read directly.

3 Plumb line

This method involves measurement of the distance from a fixed reference point to the water surface, by dropping a plumb line until it just touches the water surface.

4 Water level recorder

This instrument is used when a continuous record of water level is desired. A float and counterweight are connected by a steel tape which passes over a pulley. The float should be placed in a stilling well. A change in water level causes the pulley to rotate which, through a gearing system, moves a pen. The pen traces water level on a chart which is attached to a drum that is rotated by a clock mechanism. When properly installed and maintained, the water level recorder will provide an accurate, continuous record.

IV Units of Expression

A Volume

Preferred metric units are the cubic meter (m^3) or the liter (L). Commonly-used British units are the gallon (gal.) and the cubic foot (cu. ft., sec-ft.). Conversion factors appear below:

Multiply →	by →	To Obtain
m^3	1000	L
gal	3.79	L
cu. ft.	28.32	L
gal	0.134	cu. ft.
To Obtain ←	by ←	Divide

B **Length**

Preferred metric units are the meter (m), centimeter (cm), and millimeter (mm). Commonly-used British units are the foot (ft.) and the inch (in.). Conversion factors appear below

1 meter = 100 cm = 1000 mm

1 foot = 0.3048 meters

1 meter = 3.28 ft.

C **Flow, Flow Rate, Discharge**

All of these terms are commonly used to refer to the volume of liquid passing a point in a selected time interval. Flow is often expressed in these units.

Metric	British
m^3/sec	Gallons per minute (GPM)
L/sec	Million gallons per day (MGD)
	Cubic feet per second (cfs, sec-ft)

Conversion factors are:

Multiply ⟶	by ⟶	To Obtain
m^3/sec	1000	L/sec
GPM	0.063	L/sec
MGD	43.82	L/sec
cfs	28.32	L/sec
To Obtain	⟵ by	⟵ Divide

D Weights of constituents being carried in a liquid flow can be calculated using the following equations.

$$\text{Kg/day} = \text{MGD} \times \text{mg/L} \times 3.79$$
$$= \text{L/sec} \times \text{mg/L} \times 8.64 \times 10^{-2}$$

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This outline was prepared by F. P. Nixon Former Acting Regional Training Officer, Northeast Regional Training Center and revised by Charles E. Sponagle, OWPO, USEPA, National Training and Operational Technology Center, Cincinnati, Ohio 45268

Descriptors: Chezy Equation, Discharge Measurement, Flow, Flow Measurement, Flow Rates, Flumes, Mannings Equation, Open Channel Flow, Pipe Flow, Streamflow, Venturi Flumes, Water Flow, Water Level Recorders, Weirs

SAMPLING IN WATER QUALITY STUDIES

I INTRODUCTION

A Objective of Sampling

1 Water quality characteristics are not uniform from one body of water to another, from place to place in a given body of water, or even from time to time at a fixed location in a given body of water. A sampling program should recognize such variations and provide a basis for interpretation of their effects.

2 The purpose of collection of samples is the accumulation of data which can be used to interpret the quality or condition of the water under investigation. Ideally, the sampling program should be so designed that a statistical confidence limit may be associated with each element of data.

3 Water quality surveys are undertaken for a great variety of reasons. The overall objectives of each survey greatly influence the location of sampling stations, sample type, scheduling of sample collections, and other factors. This influence should always be kept in mind during planning of the survey.

4 The sampling and testing program should be established in accordance with principles which will permit valid interpretation.

a The collection, handling, and testing of each sample should be scheduled and conducted in such a manner as to assure that the results will be truly representative of the sources of the individual samples at the time and place of collection;

b The locations of sampling stations and the schedule of sample collections for the total sampling program should be established in such a manner that the stated investigational objectives will be met; and

c Sampling should be sufficiently repetitive over a period of time to provide valid data about the condition or quality of the water.

B Sample Variations

Interpretation of survey data is based on recognition that variations will occur in results from individual samples. While it is beyond the scope of this discussion to consider the implications of each in detail, the following can be identified as factors producing variations in data and should be considered in planning the sampling program.

1 Apparent Variations

a Variations of a statistical nature, due to collection of samples from the whole body of water, as contrasted with examination of all the water in the system.

b Variations due to inherent precision of the analytical procedures.

c Apparent variations are usually amenable to statistical analysis.

2 True Differences

a Variations of a cyclic nature

Diurnal variations, related to alternating periods of sunlight and darkness.

Diurnal variations related to waste discharges from communities.

Seasonal variations, related to temperature and its subsequent effects on chemical and biological processes and interrelationships.

Variations due to tidal influences, in coastal and estuarine waters.

b Intermittent variations

Dilution by rainfall and runoff.

Effects of irregular or intermittent discharges of wastewater, such as "slugs" of industrial wastes.

Irregular release of water from impoundments, as from power plants.

c Continuing changes in water quality

Effects downstream from points of continuous release of wastewater.

Effects of confluence with other bodies of water.

Effects of passage of the water through or over geological formations of such chemical or physical nature as to alter the characteristics of the water.

Continuing interactions of biological, physical, and chemical factors in the water, such as in the process of natural self-purification following introduction of organic contaminants in a body of water.

II LOCATION OF SAMPLING STATIONS

A The Influence of Survey Objectives

Much of the sampling design will be governed by the stated purpose of the water investigation. As an example of how different objectives might influence sampling design, consider a watercourse with points A and B located as indicated in Figure 1.

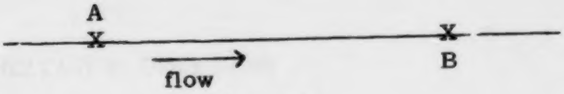

Figure 1

Point A can be the point of discharge of wastes from Community A. Point B can be any of several things, such as an intake of water treatment plant supplying Community B, or it might be the place where the river crosses a political boundary, or it may be the place where the water is subject to some legitimate use, such as for fisheries or for recreational use.

1 Assume that the objective of a water quality investigation is to determine whether designated standards of water quality are met at a water plant intake at Point B. In this case, the objective only is concerned with the quality of the water as it is available at Point B Sampling will be conducted only at Point B.

2 Alternately, consider that there is a recognized unsatisfactory water quality at Point B, and it is alleged that this is due to discharges of inadequately treated wastes, originating at Point A. Assume that the charge is to investigate this allegation.

In this case the selected sampling sites will include at least three elements:

a At least one sampling site will be located upstream from Point A, to establish base levels of water quality, and to check the possibility that the observed conditions actually originated at some point upstream from Point A.

b A site or sites must be located downstream from Point A. Such a site should be downstream a sufficient distance to permit adequate mixing in the receiving water.

c Sampling would be necessary at Point B in order to demonstrate that the water quality is in fact impaired, and that the impairment is due to influences traced from Point A.

B Hydraulic Factors

1 Flow rate and direction

a In a survey of an extended body of water it is necessary to determine the rate and direction of water movement influences selection of sampling sites. Many workers plan sampling stations representing not less than the distance water flows in a 24-hour period. Thus, in Figure 1, intervening sampling stations would be selected at points representing the distance water would flow in about 24 hours.

b In a lake or impoundment direction of flow is the major problem influencing selection of sampling stations. Frequently it is necessary to establish some sort of grid network of stations in the vicinity of the suspected sources of pollution.

c In a tidal estuary, the oscillating nature of water movement will require establishment of sampling stations in both directions from suspected sources of pollution.

2 Introduction of other water

a In situations in which a stream being studied is joined by another stream of significant size and character, sampling stations will be located immediately above the extraneous stream, in the extraneous stream above its point of juncture with the main stream, and in the main stream below the point of juncture.

b Similar stations will be needed with respect to other water discharges, such as from industrial outfalls, other communities, or other installations in which water is introduced into the main stream.

3 Mixing

a Wherever possible, one sampling point at a sample collection site is used in stream surveys. This usually is near the surface of the water, in the main channel of flow.

b In some streams mixing does not occur quickly, and introduced water moves downstream for considerable distances below the point of confluence with the main streams. Example: Susquehanna River at Harrisburg, where 3 such streams are recognizable in the main river. Preliminary survey operations should identify such situations.

When necessary, collect separate samples at two or more points across the body of water.

c Similarly, vertical mixing may not be rapid. This is noted particularly in tidal estuaries, where it may be necessary to make collections both from near the bottom and near the surface of the water.

d Collection of multiple samples from a station requires close coordination with the laboratory, in terms of the number of samples that can be examined. Some types of samples may be composited. The decision must be reached separately for each type of sample.

C Types of Analytical Procedure

1 Samples collected for physical, chemical, and bacteriological tests and measurements may be collected from the same series of sampling stations.

2 Sampling stations selected for biological (ecological) investigation require selection of a series of similar aquatic habitats (a series of riffle areas, or a series of pool areas, or both). The sites used by the aquatic biologist may or may not be compatible with those used for the rest of the survey. Accordingly, in a given stream survey, the stations used by the aquatic biologist usually are somewhat different from the stations used for other examinations.

D Access to Sampling Stations

For practical reasons, the sampling site should be easily reached by automobile if a stream survey, or by boat if the survey is on a large body of water. Highway bridges are particularly useful, if the sample collector can operate in safety.

III FACTORS IN SCHEDULING OF SAMPLING PROGRAMS

A Survey Objectives

B Time of Year

1 In short-term water quality investigations, particularly in pollution investigations, there often is need to demonstrate the extremes of pollution effects on the aquatic environment. For this reason, many short-term surveys are conducted during the warmer season of the year, at such times as the water flow rate and volume is at a minimum and there is minimum likelihood of extensive rainfall.

2 In a long-term investigation, sampling typically is conducted at all seasons of the year.

C Daily Schedules

As shown in an introductory paragraph, water quality is subject to numerous cyclic or intermittent variations. Scheduling of sample collections should be designed to reveal such variations.

1 In short-term surveys it is common practice to collect samples from each sampling site at stated intervals through the 24-hour day, continuing the program for 1 - 3 weeks. Sampling at 3-hour intervals is preferred by many workers, though practical considerations may require extension to 4- or even 6-hour intervals.

2 In an extended survey there is a tendency to collect samples from each site at not more than daily intervals, or even longer. In such cases the hour of the day should be varied through the entire program, in order that the final survey show cyclic or intermittent variations if they exist.

3 In addition, sampling in tidal waters requires consideration of tidal flows. If samples are collected but once daily, many workers prefer to make the collections at low slack tide.

4 In long-term or any other survey in which only once-daily samples are collected, it is desirable to have an occasional period of around-the-clock sampling.

IV SAMPLE COLLECTION

A Types of Samples

1 "Grab" sample - a grab sample is usually a manually collected single portion of the wastewater or stream water. An analysis of a grab sample shows the concentration of the constituents in the water at the time the sample was taken.

2 "Continuous" sample - when several points are to be sampled at frequent intervals or when a continuous record of quality at a given sampling station is required, an automatic or continuous sampler may be employed.

a Some automatic samplers collect a given volume of sample at definite time intervals, this is satisfactory when the volume of flow is constant.

b Other automatic samplers take samples at variable rates in proportion to changing rates of flow. This type of sampler requires some type of flow measuring device.

3 "Composite" sample - a composite sample is the collection and mixing together of various individual samples based upon the ratio of the volume of flow at the time the individual samples were taken to the total cumulative volume of flow. The desired composite period will dictate the magnitude of the cumulative volume of flow. The more frequently the samples are collected, the more representative will be the composite sample to the actual situation. Composite samples may be obtained by:

   a Manual sampling and volume of flow determination made when each sample is taken.

   b Constant automatic sampling (equal volumes of sample taken each time) with flow determinations made as each sample is taken.

   c Automatic sampling which takes samples at pre-determined time intervals and the volume of sample taken is proportional to the volume of flow at any given time.

B Type of Sampling Equipment

1 Manual sampling

   a Equipment is specially designed for collection of samples from the bottom muds, at various depths, or at water surfaces. Special designs are related to protection of sample integrity in terms of the water characteristic or component being measured.

   b For details of typical sampling equipment used in water quality surveys. see outlines dealing with biological, bacteriological, and chemical tests in this manual.

   c Manual sampling equipment has very broad application in field work, as great mobility of operation is possible, at lower cost than may be possible with automatic sampling equipment.

2 Automatic sampling equipment

Automatic sampling equipment has several important advantages over manual methods. Probably the most important consideration is the reduction in personnel requirements resulting from the use of this equipment. It also allows more frequent sampling than is practical manually, and eliminates many of the human errors inherent in manual sampling.

Automatic sampling equipment has some disadvantages. Probably the most important of these is the tendency of many automatic devices to become clogged when liquids high in solids are being sampled. In using automatic samplers, sampling points are fixed, which results in a certain loss of mobility as compared to manual methods.

Automatic sampling equipment should not be used indiscriminately; some types of samples - notably bacteriological, biological, and DO samples - should not be composited. In cases of doubt, the appropriate analyst should be consulted.

V SOME CONSIDERATIONS IN SAMPLING OPERATIONS

All procedures in care and handling of samples between collection and the performance of observations and tests are directed toward maintaining the reliability of the sample as an indication of the characteristics of the sample source.

A Sample Quantity

1 Samples for a series of chemical analyses require determination of the total sample volume required for all the tests, and should include enough sample in addition to provide a safety factor for laboratory errors or accidents. Many workers collect about twice the amount of sample actually required for the chemical tests. As a rule of thumb, this is on the order or 2 liters.

2 Bacteriological samples, in general, are collected in 250 - 300 ml sterile bottles; approximately 150 - 200 ml of samples is adequate in practically all cases.

B Sample Identification

1 Sample identification must be maintained throughout any survey. It is vital, therefore, that adequate records be made of all information relative to the source of the sample and conditions under which the collection was made. All information must be clearly understandable and legible.

2 Every sample should be identified by means of a tag or bottle marking, firmly affixed to the sample bottle. Any written material should be with indelible marking material.

3 Minimum information on the sample label should include identification of the sample site, date and time of collection, and identification of the individual collecting the sample.

4 Supplemental identification of samples is strongly recommended, through maintenance of a sample collection logbook. If not included on the sample tag (some prefer to duplicate such information) the logbook can show not only the sample site and date and hour of collection, but also the results of any tests made on site (such as temperature, pH, dissolved oxygen). In addition, the logbook should provide for notation of any unusual observations made at the sampling site, such as rainfall, direction and strength of unusual winds, or evidence of disturbance of the collection site by human or other animal activity.

C Care and Handling of Samples

1 As a general policy, all observations and tests should be made as soon as possible after sample collection.

a Some measurements require performance at the sampling site, such as temperature, pH, dissolved oxygen, chlorine, flow rate, etc.

b Some tests are best made at the sampling site because the procedures are simple, rapid, and of acceptable accuracy. These may include such determinations as conductivity.

c Some additional determinations, such as alkalinity, hardness, and turbidity may be made in the field, provided that ease, convenience, and reliability of results are acceptable for the purposes of the study

2 Samples to be analyzed in the laboratory require special protection to assure that the quality measured in the sample represents the condition of the source. Many samples, especially those subjected to biological analysis, require special preservation, protection, and handling procedures. In case of doubt, the appropriate analyst should be consulted. Most common procedures for sample protection include:

a Examination within brief time after collection.

b Temperature control.

c Protection from light.

d Addition of preservative chemicals.

Applications of these sample protective procedures are along the following lines:

3 Early examination of sample

Applicable to all types of samples.

4 Temperature control

a All biological materials for examination in a living state should be iced between collection and examination.

b Bacteriological samples should be iced during a maximum transport time of 6 hours. Such samples should be refrigerated upon receipt in the laboratory and processed within 2 hours.

c Chemical samples often require icing

Preservation by refrigeration at 4°C is recommended for acidity, alkalinity, BOD, color, sulfate, threshold odor, and other samples. Holding times vary Quick freezing will permit retention of many samples for up to several months prior to laboratory examination.

5 Protection from light

a Any constituent of water which may be influenced by physiochemical reactions involving light should be protected. DO samples brought to the iodine stage, for example, should be protected from light prior to titration.

b In addition, any water constituent (such as dissolved oxygen) which may be influenced by algal activity should be protected from light.

6 Addition of chemical preservatives

a Bacteriological samples never should be "protected" by addition of preservative agents. The only permissible chemical additive is sodium thiosulfate, which is used to neutralize free residual chlorine, if present, and which is added to the sampling bottle before sterilization.

b Samples for biological examination should be protected by chemical additives only under specific direction of the principal biologist in a water quality study.

c For chemical tests, preservatives are useful for a number of water components. Specific instructions for the preservation of a number of chemical constituents are given in reference (4).

1 Standard Methods for the Examination of Water and Wastewater. APHA. 14th Ed. 1975

2 Planning and Making Industrial Waste Surveys. Ohio River Valley Water Sanitation Commission.

3 Industry's Idea Clinic. Journal of the Water Pollution Control Federation April, 1965.

4 Methods for Chemical Analysis of Water and Wastes, 1979. USEPA, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

This outline was prepared by H. L. Jeter, Former Director, National Training Center, USEPA, OWPO, Cincinnati, Ohio 45268 and P. F. Atkins, Jr., former Sanitary Engineer, Training Activities, and revised by Charles E. Sponagle Sanitary Engineer, National Training and Operational Techology Center, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Instrumentation, On-Site Investigations, Preservation, Samplers, Sampling, Water Sampling, Handling, Sample, Surface Waters, Wastewater

SAMPLE HANDLING - FIELD THROUGH LABORATORY

I PLANNING A SAMPLING PROGRAM

A Factors to Consider:

1 Locating sampling sites

2 Sampling equipment

3 Type of sample required

a grab
b composite

4 Amount of sample required

5 Frequency of collection

6 Preservation measures, if any

B Decisive Criteria

1 Nature of the sample source

2 Stability of constituent(s) to be measured

3 Ultimate use of data

II REPRESENTATIVE SAMPLES

If a sample is to provide meaningful and valid data about the parent population, it must be representative of the conditions existing in that parent source at the sampling location.

A The container should be rinsed two or three times with the water to be collected.

B Compositing Samples

1 For some sources, a composite of samples is made which will represent the average situation for stable constituents.

2 The nature of the constituent to be determined may require a series of separate samples.

C The equipment used to collect the sample is an important factor to consider ASTM(1) has a detailed section on various sampling devices and techniques.

D Great care must be exercised when collecting samples in sludge or mud areas and near benthic deposits. No definite procedure can be given, but careful effort should be made to obtain a representative sample.

III SAMPLE IDENTIFICATION

A Each sample must be unmistakably identified, preferably with a tag or label. The required information should be planned in advance.

B An information form preprinted on the tags or labels provides uniformity of sample records, assists the sampler, and helps ensure that vital information will not be omitted.

C Useful Identification Information includes:

1 sample identity code
2 signature of sampler
3 signature of witness
4 description of sampling location detailed enough to accommodate reproducible sampling. (It may be more convenient to record the details in the field record book).
5 sampling equipment used
6 date of collection
7 time of collection
8 type of sample (grab or composite)
9 water temperature
10 sampling conditions such as weather, water level, flow rate of source, etc.
11 any preservative additions or techniques
12 record of any determinations done in the field
13 type of analyses to be done in laboratory

IV SAMPLE CONTAINERS

A Available Materials

1 glass
2 plastic
3 hard rubber

B Considerations

1 Nature of the sample - Organics attack polyethylene.

2 Nature of constituent(s) to be determined - Cations can adsorb readily on some plastics and on certain glassware. Metal or aluminum foil cap liners can interfere with metal analyses.

3 Preservatives to be used - Mineral acids attack some plastics.

4 Mailing Requirements - Containers should be large enough to allow extra volume for effects of temperature changes during transit. All caps should be securely in place. Glass containers must be protected against breakage. Styrofoam linings are useful for protecting glassware.

C Preliminary Check

Any question of possible interferences related to the sample container should be resolved before the study begins. A preliminary check should be made using corresponding sample materials, containers, preservatives and analysis.

D Cleaning

If new containers are to be used, preliminary cleaning is usually not necessary.

If the sample containers have been used previously, they should be carefully cleaned before use.

There are several cleaning methods available. Choosing the best method involves careful consideration of the nature of the sample and of the constituent(s) to be determined.

1 Phosphate detergents should not be used to clean containers for phosphorus samples.

2 Traces of dichromate cleaning solution will interfere with metal analyses.

E Storage

Sample containers should be stored and transported in a manner to assure their readiness for use.

V SAMPLE PRESERVATION

Every effort should be made to achieve the shortest possible interval between sample collection and analyses. If there must be a delay and it is long enough to produce significant changes in the sample, preservation measures are required.

At best, however, preservation efforts can only retard changes that inevitably continue after the sample is removed from the parent population.

A Functions

Methods of preservation are relatively limited. The primary functions of those employed are:

1 to retard biological action
2 to retard precipitation or the hydrolysis of chemical compounds and complexes
3 to reduce volatility of constituents

B General Methods

1 pH control - This affects precipitation of metals, salt formation and can inhibit bacterial action.

2 Chemical Addition - The choice of chemical depends on the change to be controlled.

Mercuric chloride is commonly used as a bacterial inhibitor. Disposal of the mercury-containing samples is a problem and efforts to find a substitute for this toxicant are underway.

To dispose of solutions of inorganic mercury salts, a recommended procedure is to capture and retain the mercury salts as the sulfide at a high pH. Several firms have tentatively agreed to accept the mercury sulfide for re-processing after preliminary conditions are met.(4)

3 Refrigeration and Freezing - This is the best preservation technique available, but it is not applicable to all types of samples. It is not always a practical technique for field operations.

C Specific Methods

The EPA Methods Manual(2) includes a table summarizing the holding times and preservation techniques for several analytical procedures. This information also can be found in the standard references(1, 2, 3) as part of the presentation of the individual procedures.

D Federal Register Methods

When collecting samples to be analyzed for National Pollutant Discharge Elimination System or State Certification report purposes, one must consult the appropriate Federal Register(5) for information about sample handling procedures. When collecting samples to be analyzed for compliance with maximum contaminant levels in drinking water, consult the EPA Report(6) which includes this information.

VI METHODS OF ANALYSIS

Standard reference books of analytical procedures to determine the physical and chemical characteristics of various types of water samples are available.

A EPA Methods Manual

The Environmental Monitoring and Support Laboratory of the Environmental Protection Agency, has published a manual of analytical procedures to provide methodology for monitoring the quality of our Nation's Waters and to determine the impact of waste discharges. The title of this manual is "Methods for Chemical Analysis of Water and Wastes."(2)

For some tests, this manual refers the analyst to Standard Methods and/or to ASTM for the stepwise procedure.

B Standard Methods

The American Public Health Association, the American Water Works Association and the Water Pollution Control Federation prepare and publish a volume describing methods of water analysis. These include physical and chemical procedures. The title of this book is "Standard Methods for the Examination of Water and Wastewater."(3)

C ASTM Standards

The American Society for Testing and Materials publishes an annual "book" of specifications and methods for testing materials. The "book" currently consists of 47 parts. The part applicable to water is a book titled, "Annual Book of ASTM Standards", Part 31, Water.(1)

D Other References

Current literature and other books of analytical procedures with related information are available to the analyst.

E Federal Register Methodology

The analyst must consult the appropriate Federal Register for a listing of approved methodology if he is gathering data for National Pollutant Discharge Elimination System(5) or State Certification(5) report purposes, or to document compliance with maximum contaminant levels in drinking water(7). The Federal Register directs the user to pages in the above cited reference books where acceptable procedures can be found. The Federal Register also provides information concerning the protocol for obtaining approval to use analytical procedures other than those listed.

VII ORDER OF ANALYSES

The ideal situation is to perform all analyses shortly after sample collection. In the practical order, this is rarely possible. The allowable holding time for preserved samples is the basis for scheduling analyses.

A The allowable holding time for samples depends on the nature of the sample, the stability of the constituent(s) to be determined and the conditions of storage.

1 For some constituents and physical values, immediate determination is required, e.g. dissolved oxygen, pH.

2 Using preservation techniques, the holding times for other determinations range from 24 hours (BOD) to 7 days (COD). Metals may be held up to 6 months.(2)

3 The EPA Methods Manual(2) and Standard Methods(3) include a table summarizing holding times and preservation techniques for several analytical procedures. Additional information can be found in the standard references(1, 2, 3) as part of the presentation of the individual procedures.

4 A table with proposed holding times and preservation techniques applicable to samples collected for National Pollutant Discharge Elimination System or State Certification purposes was published in the December 18, 1979 Register(5). A similar table for drinking water samples can be found in a May, 1978 Report(6).

5 If dissolved concentrations are sought, filtration should be done in the field if at all possible. Otherwise, the sample is filtered as soon as it is received in the laboratory. A 0.45 micrometer membrane filter is recommended for reproducible filtration.

B The time interval between collection and analysis is important and should be recorded in the laboratory record book.

VIII RECORD KEEPING

The importance of maintaining a bound, legible record of pertinent information on samples cannot be over-emphasized.

A Field Operations

A bound notebook should be used. Information that should be recorded includes·

1 Sample identification records (See Part III)

2 Any information requested by the analyst as significant

3 Details of sample preservation

4 A complete record of data on any determinations done in the field. (See B, next)

5 Shipping details and records

B Laboratory Operations

Samples should be logged in as soon as received and the analyses performed as soon as possible.

A bound notebook should be used. Preprinted data forms provide uniformity of records and help ensure that required information will be recorded. Such sheets should be permanently bound.

Items in the laboratory notebook would include:

1 sample identifying code
2 date and time of collection
3 date and time of analysis
4 the analytical method used
5 any deviations from the analytical method used and why this was done
6 data obtained during analysis
7 results of quality control checks on the analysis
8 any information useful to those who interpret and use the data
9 signature of the analyst

IX SUMMARY

Valid data can be obtained only from a representative sample, unmistakably identified, carefully collected and stored. A skilled analyst, using approved methods of analyses and performing the determinations within the prescribed time limits, can produce data for the sample. This data will be of value only if a written record exists to verify sample history from the field through the laboratory.

REFERENCES

1 ASTM Annual Book of Standards, Part 31, Water, 1975.

2 Methods for Chemical Analysis of Water and Wastes, EPA-EMSL, Cincinnati, Ohio 45268, 1979.

3 Standard Methods for the Examination of Water and Wastewater, 14th edition APHA-AWWA-WPCF, 1975.

4 Dean, R., Williams, R. and Wise, R., Disposal of Mercury Wastes from Water Laboratories, Environmental Science and Technology, October, 1971.

5 Federal Register, "Guidelines Establishing Test Procedures for the Analysis of Pollutants," Vol. 41, No. 232, December 1, 1976, pp 52780-52786. Also, Vol. 44, No. 244, December 18, 1979 pp 75028-75052 presents proposed changes. The latter is scheduled for finalization after January, 1981.

6 "Manual for the Interim Certification of Laboratories Involved in Analyzing Public Drinking Water Supplies - Criteria and Procedures," U. S. EPA Report No. EPA 600/8-78-008, May, 1978.

7 Federal Register, "National Interim Primary Drinking Water Regulations," Vol. 40, No. 248, December 24, 1975, pp 59566-59574. Also, "Interim Primary Drinking Water Regulations; Amendments", Vol. 45, No. 168, August 27, 1980, pp 57332-57346.

This outline was prepared by Audrey Kroner, Chemist, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268.

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COLLECTION AND HANDLING OF SAMPLES FOR BACTERIOLOGICAL EXAMINATION

I INTRODUCTION

The first step in the examination of a water supply for bacteriological examination is careful collection and handling of samples. Information from bacteriological tests is useful in evaluating water purification, bacteriological potability, waste disposal, and industrial supply. Topics covered include: representative site selection, frequency, number, size of samples, satisfactory sample bottles, techniques of sampling, labeling, and transport.

II SELECTION OF SAMPLING LOCATIONS

The basis for locating sampling points is collection of representative samples.

A Take samples for potability testing from the distribution system through taps. Choose representative points covering the entire system. The tap itself should be clean and connected directly into the system. Avoid leaky faucets because of the danger of washing in extraneous bacteria. Wells with pumps may be considered similar to distribution systems.

B Grab samples from streams are frequently collected for control data or application of regulatory requirements. A grab sample can be taken in the stream near the surface.

C For intensive stream studies on source and extent of pollution, representative samples are taken by considering site, method and time of sampling. The sampling sites may be a compromise between physical limitations of the laboratory, detection of pollution peaks, and frequency of sample collection in certain types of surveys. First, decide how many samples are needed to be processed in a day. Second, decide whether to measure cycles of immediate pollution or more average pollution.

Sites for measuring cyclic pollution are immediately below the pollution source. Sampling is frequent, for example, every three hours.

A site designed to measure more average conditions is far enough downstream for a complete mixing of pollution and water. Keep in mind that averaging does not remove all variation but only minimizes sharp fluctuations. Downstream sites sampling may not need to be so frequent

Samples may be collected 1/4, 1/2 and 3/4 of the stream width at each site or other distances, depending on survey objectives. Often only one sample in the channel of the stream is collected. Samples are usually taken near the surface

D Samples from lakes or reservoirs are frequently collected at the drawoff and usually about the same depth and may be collected over this entire surface.

E Collect samples of bathing beach water at locations and times where the most bathers swim.

III NUMBER, FREQUENCY AND SIZE OF SAMPLES

A For determining sampling frequency for drinking water, consult the USEPA Standards.

1 The total number, frequency, and site are established by agreement with either state of USEPA authorities.

2 The minimum number depends upon the number of users. Figure 1 indicates that the smaller populations call for relatively more samples than larger ones. The numbers on the left of the graph refer to actual users and not the population shown by census.

3 In the event that coliform limits of the standard are exceeded, daily samples must be taken at the same site. Examinations should continue until two consecutive samples show coliform level is satisfactory. Such samples are to be considered as special samples and shall not be included in the total number of samples examined.

4 Sampling programs described above represent a minimum number which may be increased by reviewing authority.

B For stream investigations the type of study governs frequency of sampling.

C Collect swimming pool samples when use is heavy. The high chlorine level rapidly reduces the count when the pool is not in use. Residual chlorine tests are necessary to check neutralization of chlorine in the sample.

D Lake beaches may be sampled as required depending on the water uses.

Population served:	Minimum number of samples per month
25 to 1,000	1
1,001 to 2,500	2
2,501 to 3,300	3
3,301 to 4,100	4
4,101 to 4,900	5
4,901 to 5,800	6
5,801 to 6,700	7
6,701 to 7,600	8
7,601 to 8,500	9
8,501 to 9,400	10
9,401 to 10,300	11
10,301 to 11,100	12
11,101 to 12,000	13
12,001 to 12,900	14
12,901 to 13,700	15
13,701 to 14,600	16
14,601 to 15,500	17
15,501 to 16,300	18
16,301 to 17,200	19
17,201 to 18,100	20
18,101 to 18,900	21
18,901 to 19,800	22
19,801 to 20,700	23
20,701 to 21,500	24
21,501 to 22,300	25
22,301 to 23,200	26
23,201 to 24,000	27
24,001 to 24,900	28
24,901 to 25,000	29
25,001 to 28,000	30
28,001 to 33,000	35
33,001 to 37,000	40
37,001 to 41,000	45
41,001 to 46,000	50
46,001 to 50,000	55
50,001 to 54,000	60
54,001 to 59,000	65
59,001 to 64,000	70
64,001 to 70,000	75
70,001 to 76,000	80
76,001 to 83,000	85
83,001 to 90,000	90
90,001 to 96,000	95
96,001 to 111,000	100
111,001 to 130,000	110
130,001 to 160,000	120
160,001 to 190,000	130
190,001 to 220,000	140
220,001 to 250,000	150
250,001 to 290,000	160
290,001 to 320,000	170
320,001 to 360,000	180
360,001 to 410,000	190
410,001 to 450,000	200
450,001 to 500,000	210
500,001 to 550,000	220
550,001 to 600,000	230
600,001 to 720,000	240
720,001 to 780,000	250
780,001 to 840,000	260
840,001 to 910,000	270
910,001 to 970,000	280
970,001 to 1,050,000	290
1,050,001 to 1,140,000	300
1,140,001 to 1,230,000	310
1,230,001 to 1,320,000	320
1,320,001 to 1,420,000	330
1,420,001 to 1,520,000	340
1,520,001 to 1,630,000	350
1,630,001 to 1,730,000	360
1,730,001 to 1,850,000	370
1,850,001 to 1,970,000	380
1,970,001 to 2,060,000	390
2,060,001 to 2,270,000	400
2,270,001 to 2,510,000	410
2,510,001 to 2,750,000	420
2,750,001 to 3,020,000	430
3,020,001 to 3,320,000	440
3,320,001 to 3,620,000	450
3,620,001 to 3,960,000	460
3,960,001 to 4,310,000	470
4,310,001 to 4,690,000	480
4,690,001 or more	500

FIGURE I

E Salt water or estuarine beaches are sampled as needed with frequency depending on use.

F Size of samples depends upon examination anticipated. Generally 100 ml is the minimum size.

IV BOTTLES FOR WATER SAMPLES

A The sample bottles should have capacity for at least 100 ml of sample, plus an air space. The bottle and cap must be of bacteriological inert materials. Resistant glass or heat resistant plastic are acceptable. At the National Training Center, wide mouth ground-glass stoppered bottles (Figure 2) are used.

All bottles must be properly washed and sterilized. Protect the top of the bottles and cap from contamination by paper or metal foil hoods. Both glass and heat resistant plastic bottles may be sterilized in an autoclave. Hold plastic at 121°C for at least 10 minutes. Hot air sterilization. 2 hours at 170°C, may be used for dry glass bottles.

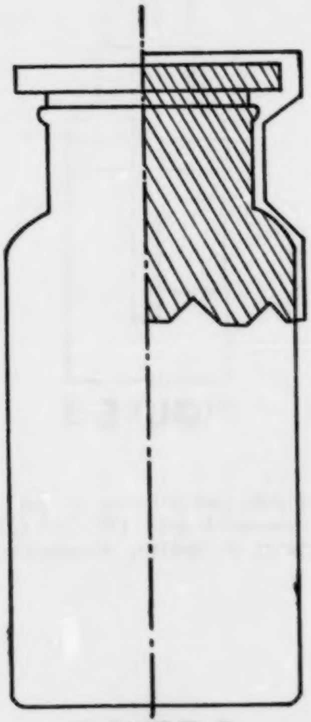

FIGURE 2

B Add sodium thiosulfate to bottles intended for halogenated water samples. A quantity of 0.1 ml of a 10% solution provides 100 mg per liter concentration in a 100 ml sample. This level shows no effect upon viability or growth.

C Supply catalogs list wide mouth ground glass stoppered bottles of borosilicate resistance glass, specially for water samples.

V TECHNIQUE OF SAMPLE COLLECTION

Follow aseptic technique as nearly as possible. Nothing but sample water must touch the inside of the bottle or cap. To avoid loss of sodium thiosulfate, fill the bottle directly and do not rinse. Always remember to leave an air space.

A In sampling from a distribution system, first run the faucet wide open until the service line is cleared. A time of 2-3 minutes generally is sufficient. Reduce the flow and fill the sample bottle without splashing. Some authorities stress flaming the tap before collection, but the use of this technique is now generally considered as valueless. A chlorine determination is often made on the site.

B The bottle may be dipped into some waters by hand. Avoid introduction of bacteria from the human hand and from surface debris. Some suggestions follow· Hold the bottle near the base with one hand and with the other remove the hood and cap. Push the bottle rapidly into the water mouth down and tilt up up towards the current to fill. A depth of about 15 cm (6 inches) is satisfactory. When there is no current move the bottle through the water horizontally and away from the hand. Lift the bottle from the water, having left an air space of about 2½ cm (1 inch), and return the uncontaminated cap.

C Samples may be dipped from swimming pools. Determine residual chlorine on the pool water at the site. Test the sample at the laboratory to check chlorine neutralization by the thiosulfate.

D Sample bathing beach water by wading out to the 60 cm (2 ft) depth and dipping the sample up from about 15 cm (6 inches) below the surface. Use the procedure described in V. B.

E Wells with pumps are similar to distribution systems. With a hand pumped well, waste water for about five minutes before taking the sample. Sample a well without a pump by lowering a sterile bottle attached to a weight. A device which opens the bottle underneath the water will avoid contamination by surface debris.

F Various types of sampling devices are available where the sample point is inaccessible or depth samples are desired. The general problem is to put a sample bottle in place, open it, close it, and return it to the surface. No bacteria but those in the sample must enter the bottle.

1 The J - Z sampler described by Zobell in 1941, was designed for deep sea sampling but is useful elsewhere (Figure) 3). It has a metal frame, breaking device for a glass tube, and sample bottle. The heavy metal messenger strikes the lever arm which breaks the glass tubing at a file mark. A bent rubber tube straightens and the water is drawn in several inches from the apparatus. Either glass or collapsible rubber bottles are sample containers.

Commercial adaptations are available.

2 Note the vane and lever mechanism on the New York State Conservation Department's sampler in Figure 4. When the apparatus is at proper depth the suspending line is given a sharp pull. Water inertia against the vane raises the stopper and water pours into the bottle. Sufficient sample is collected prior to the detachment of the stopper from the vane arm allowing a closure of the sample bottle.

The New York State Conservation Department's sampler is useful for shallow depths and requires nothing besides glass stoppered sample bottles.

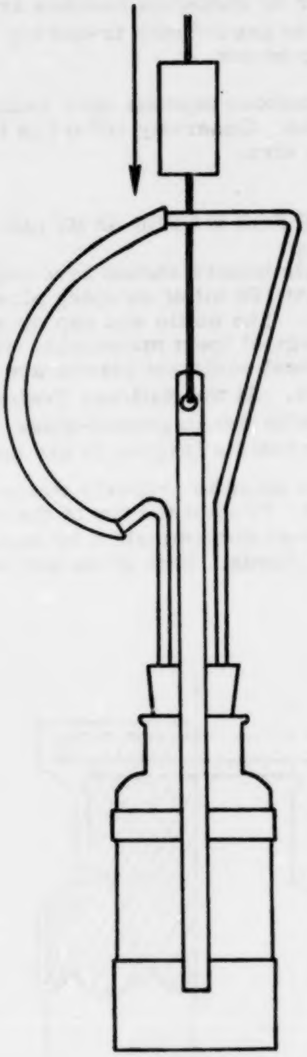

FIGURE 3

Reproduced with permission of the Journal of Marine Research 4:3, 173-188 (1941) by the Department of Health, Education and Welfare.

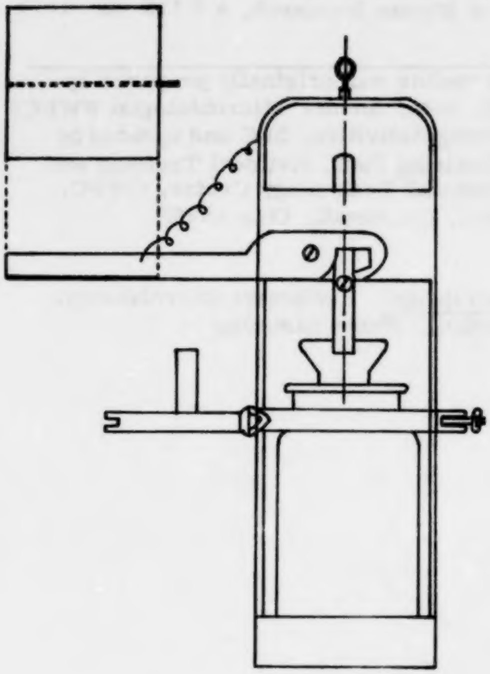

FIGURE 4

3 A commercial sampler is available which is an evacuated sealed tube with a capillary tip. When a lever on the support rack breaks the tip, the tube fills. Other samplers exist with a lever for pulling the stopper, while another uses an electromagnet.

VI DATA RECORDING

A Information generally includes: date, time of collection, temperature of water, locatio of sampling point, and name of the sample collector. Codes are often used. The location description must be exact enough to guide another person to the site. Reference to bridges, roads, distance to the nearest town may help. Use of the surveyors' description and maps are recommended. Mark identification on the bottles or on securely fastened tags. Gummed tags may soak off and are inadvisable.

B While a sanitary survey is an indispensable part of the evaluation of a water supply, its discussion is not within the scope of this lecture. The sample collector could supply much information if desired.

VI SHIPPING CONDITIONS

The examination should commence as soon as possible, preferably within one hour. A maximum elapsed time between collection and examination is 30 hours for potable water samples and 6 hours for other water samples (time from collection to laboratory delivery). An additional 2 hours is allowed from delivery to laboratory to the completion of first-day laboratory procedures. Standard Methods (14th Edition) recommends icing of samples between collection and testing.

VII PHOTOGRAPHS

A photograph is a sample in that it is evidence representing water quality. Sample collectors and field engineers may carry cameras to record what they see. Pictures help the general public and legal courts to better understand laboratory data.

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This outline was originally prepared by A. G. Jose, former Microbiologist FWPCA Training Activities, SEC and updated by the Training Staff, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Equipment Microbiology, Sampling, Water Sampling

BIOLOGICAL FIELD METHODS

I INTRODUCTION

A Due to the nature of ecological interrelationships, methods for the collection of different types of aquatic organisms differ. In general we can recognize those that swim or float and those that crawl, those that are big and those that are little. Each comprises a part of "the life" at any given survey station and consequently a "complete" collection would include all types.

B Field methods in the following outline are grouped under four general categories, the collection of:

1 Benthos (or bottom dwelling organisms). These may be attached, crawling, or burrowing forms.

2 Plankton (plancton). These are usually microscopic plants and animals feebly swimming, drifting, or suspended in the open water.

3 Periphyton or "aufwuchs". This is the community of organisms associated with submersed substrates. Some are attached, some crawl. The group is intermediate between the benthos and the plankton.

4 Nekton. Nekton are the larger, free swimming active animals such as shrimp or fishes.

C Aquatic mammals, birds, amphibians, reptiles, and in most cases, require still other approaches and are not included.

D There is little basic difference between biological methods for oceanic, estuarine, or freshwater situations except those dictated by the physical nature of the environments and the relative sizes of the organisms. Fish, benthos, and plankton collection is essentially the same whether conducted in Lake Michigan, Jones' Beach, or the Sargasso Sea.

1 Marine organisms range to larger sizes, and the corrosive nature of seawater dictates special care in the design and maintenance of marine equipment. Site selection and collection schedules are influenced by such factors as tidal currents and periodicity, and salinity distribution, rather than (river) currents, riffles, and pools.

2 Freshwater organisms are in general smaller, and the water is seldom chemically corrosive on equipment. Site selection in streams involves riffles, falls, pools, etc., and a unidirectional flow pattern. Lake collection may involve less predictable stratification or flow patterns.

E Definite objectives should be established in advance as to the size range of organisms to be collected and counted, i.e.: microscopic only, microscopic and macroscopic, those retained by "30 mesh" screens, invertebrates and/or vertebrates, etc.

II STANDARD PROCEDURES

A Certain standard supplementary procedures are a part of all field techniques. In order to be interpreted and used, every collection must be associated with a record of environmental conditions at the time of collection.

1 Data recorded should include the following as far as practicable.

Location (name of river, lake, etc.) Marked USGS $7\frac{1}{2}$ minute quadrangles are recommended.

Station number (particular location of which a full description should be on record)

Date and hour

Weather

Air temperature

Wind direction and velocity

Sky or cloud cover

Water temperature (at various depths, if applicable)

Salinity (at various depths, if applicable)

Tidal flow (ebb or flood)

Turbidity (or light penetration, etc.)

Water color

Depth

Type of bottom

Type of collecting device and accessories

Method of collecting

Type of sample (quantitative or qualitative)

Number of samples at each station

Chemical and physical data, e.g., (replicates and stratification) dissolved oxygens nutrients pH, etc.

Collector's name

Miscellaneous observations (often very important)

2 All collecting containers should be identified at least with location, station number, sample number, and date. Spares are very handy.

3 Much transcription of data can be eliminated by using sheets or cards with a uniform arrangement for including the above data. The same field data sheet may include field or laboratory analysis.

B Compact kits of field collecting equipment and materials greatly increase collecting efficiency, especially if the collection site is remote from transportation.

III PERSONAL OBSERVATION AND PHOTOGRAPHY

A Direct or indirect observation of underwater conditions has become relatively efficient.

1 Submersibles are proving very important for deep water observations.

2 Use of aqualung permits direct personal study down to over 200 feet.

3 Underwater television (introduced by the British Admiralty for military purposes) is now generally available for biological and other observations.

4 Underwater photography is improving in quality and facility.

5 Underwater swimming or use of SCUBA is quite valuable for direct observation and collecting.

6 Smaller hand held water lens or water telescopes are very useful in shallow water.

IV COLLECTION OF BOTTOM OR BENTHIC ORGANISMS

A Shoreline or Wading Depth Collecting Plates I, II

1 Hand picking of small forms attached to or crawling off rocks, sticks, etc. when lifted out of the water is a

fundamental and much used method for quickly assaying what is present and what may be expected on further search.

2 Patches of seaweed and eelgrass and shallow weedy margins anywhere are usually studied on a qualitative basis only.

  a The apron net is one of the best tools for animals in weed beds or other heavy vegetation. It is essentially a pointed wire sieve on a long handle with coarse screening over the top to keep out leaves and sticks.

  b Grapple hooks or a rake may be used to pull masses of vegetation out on the bank where the fauna may be examined and collected as they crawl out.

  c Quantitative estimates of both plants and animals can be made with a "stove pipe" sampler which is forced down through a weed mass in shallow water and embedded in the bottom. Entire contents can then be bailed out into a sieve and sorted.

  d A frame of known dimensions may be placed over an area to be sampled and the material within cropped out. This is especially good for larger plants and large bivalves. This method yields quantitative data.

3 Sand and mud flats in estuaries and shallow lakes may be sampled quantitatively by marking off a desired area and either digging away surrounding material or excavating the desired material to a measured depth. Handle-operated samplers recently developed by Jackson and Larrimore, make for more effective sampling of a variety of bottoms down to the depth of the handles. Such samples are then washed through graded screens to retrieve the organisms.

4 **Ekman grabs** are most useful on soft bottoms. This is a completely closing clamshell type grab with spring operated jaws. Size of grab is usually 15.2 x 15.2 cm or 22.9 x 22.9, the 30.5 x 30.5 cm size is impractical due to its heavy weight when filled with bottom material.

For use in shallow water, it is convenient to rig an Ekman with a handle and a hand operated jaw release mechanism.

The Ekman may also be fitted with a hydraulic operated closing mechanism for "hard" substrates.

5 The Petersen type grab (described below) without weights will take satisfactory samples in firm muds, but tends to bury itself in very soft bottoms. It is seldom used in shallow water except as noted below.

B Collecting in Freshwater Riffles or Rapids

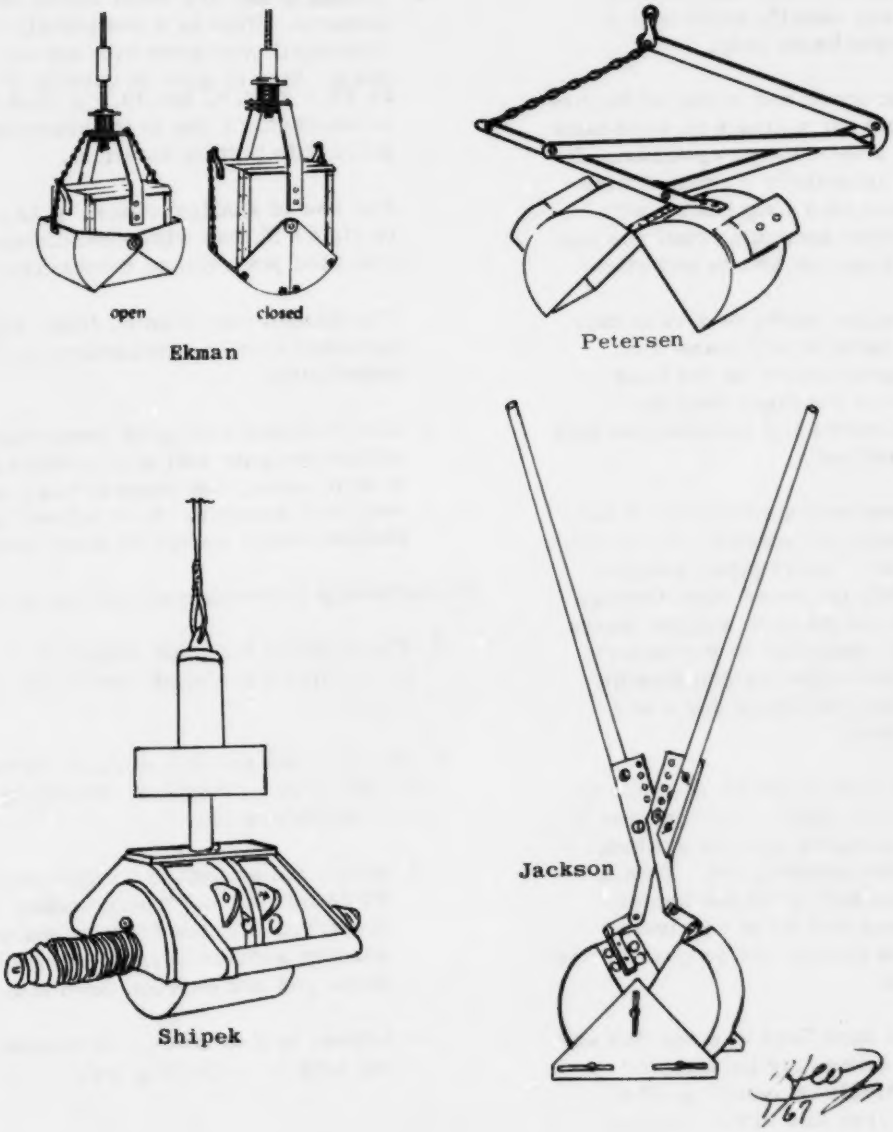
1 The riffle is the most satisfactory habitat for comparing stream conditions at different points.

2 The hand screen is a simple and easy device to use in this situation. Resulting collections are qualitative only.

  a In use the screen is firmly planted in the stream bed. Upstream bottom is thoroughly disturbed with the feet, or worked over by hand by another person. Organisms dislodged are carried down into the screen.

  b Screen is then lifted and dumped into sorting tray or collecting jar.

BOTTOM GRABS



open

closed

Ekman

Petersen

Shipek

Jackson

PLATE I

LIMNOLOGICAL EQUIPMENT

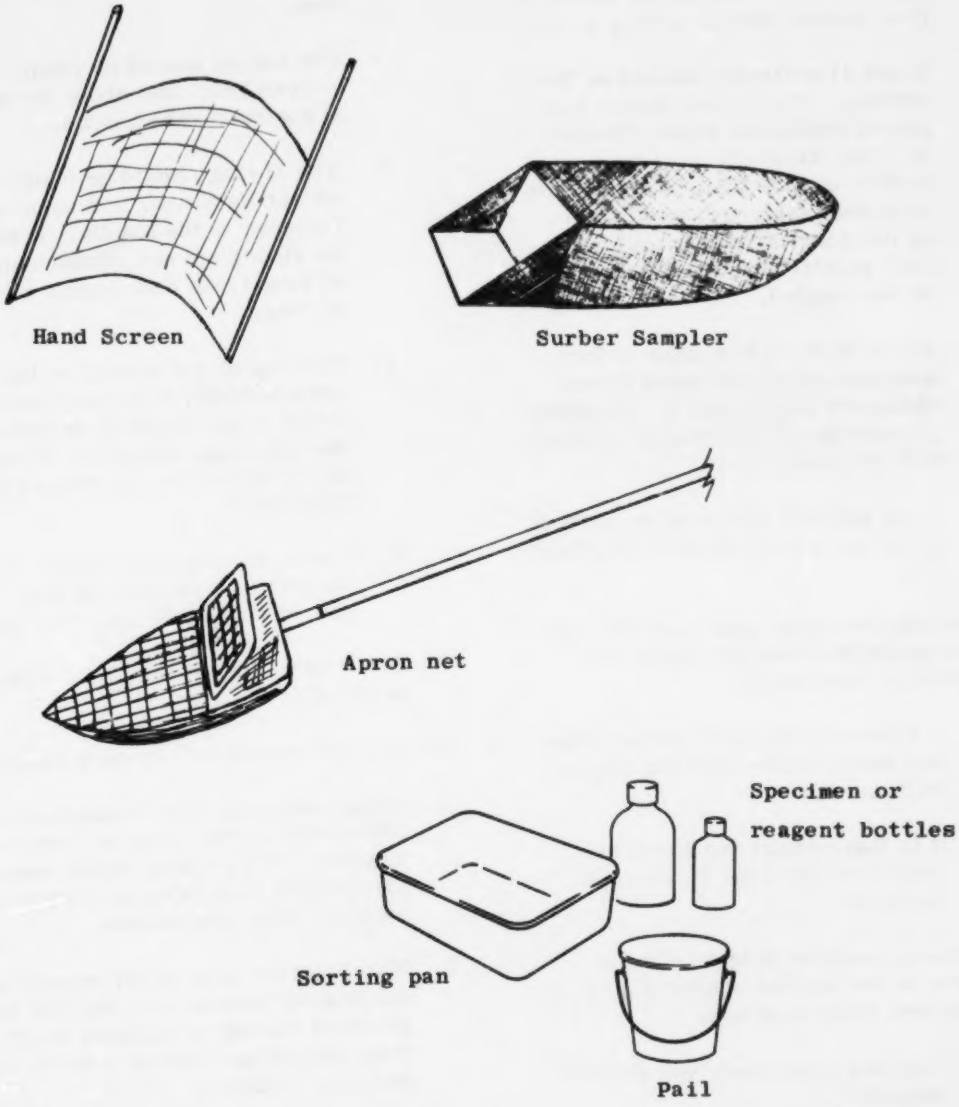

Hand Screen
Surber Sampler
Apron net
Specimen or
reagent bottles
Sorting pan
Pail

PLATE II

3 The well-known Surber sampler is one of the best quantitative collecting devices for riffles.

  a It consists of a frame 30.5 cm square with a conical net attached. It is usable only in moving water.

  b In use it is firmly planted on the bottom. The bottom stones and gravel within the square frame are then carefully gone over by hand to ensure that all organisms have been dislodged and carried by the current into the net. A stiff vegetable brush is often useful in this regard.

  c From three to five square-foot samples should be taken at each station to insure that a reasonable percentage of the species present will be represented.

  d Long sleeved rubber gloves (trappers) are recommended for winter use.

4 The Petersen type grab may be used in deep swift riffles or where the Surber is unsuitable.

  a It is planted by hand on the bottom, and worked down into the bottom with the feet.

  b It is then closed and lifted by pulling on the rope in the usual manner.

5 A strong medium weight D frame dipnet is the closest approach to a universal collecting tool.

  a Sweeping Weed beds and Stream Margins

  This is used with a sweeping motion, through weeds, over the bottoms or in open water. A triangular shape is preferred by some.

  b Stop net or Kicking Technique

  This may be used as a roughly quantitative device in riffles by holding the end flat against the bottom and disturbing the substrate with one's feet. A standard period of time is used.

  c The handle should be from 1 to 2 meters long, and about the weight of a garden rake handle.

  d The D ring should be made of steel or spring brass, and securely fastened to the handle. It should be strong but not cumbersome; size of ring stock will depend on width of ring.

  e The bag or net should be the strongest available, not over 3 mm mesh. Avoid mesh which is so fine that the net plugs easily and is slow and heavy to handle. A shallow bag is preferred.

  f A wide canvas apron sewed around the rim will protect the bag. The rim may be protected with leather.

6 Drift nets are set for predetermined periods.

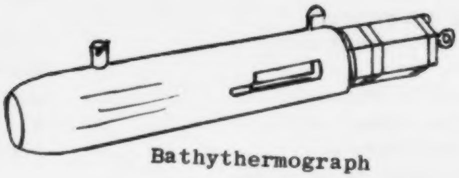
D Deep Water Benthic Collecting Plate III

1 When sampling from vessels, a crane and winch, either hand or power operated, is used. The general ideas described for shallow waters apply also to deeper waters, when practicable.

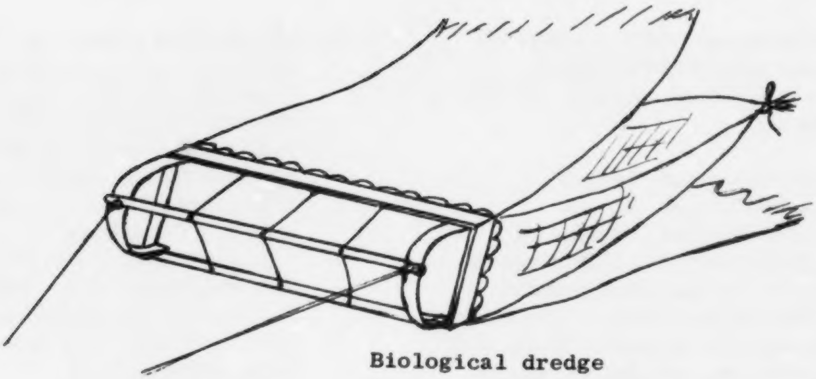
2 The Petersen type grab, seems to be the best all around sampler for the greatest variety of bottoms at all depths, from shoreline down to over 10,000 meters. (Plate I)

  a It consists of two heavily constructed half cylinders closed together by a strong lever action.

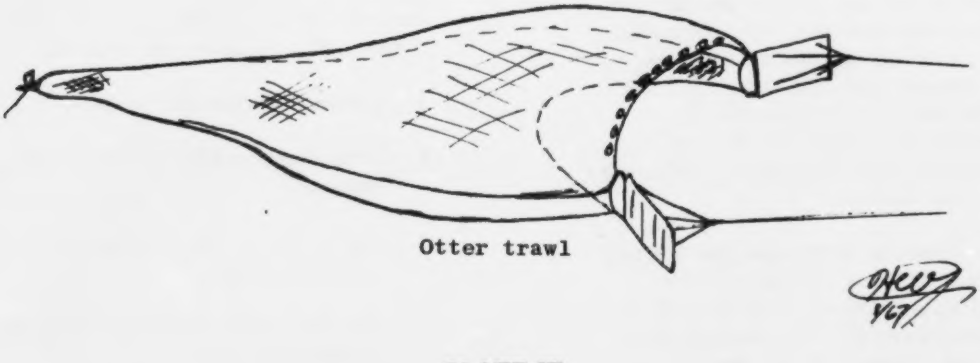
DEEP WATER EQUIPMENT



Bathythermograph



Biological dredge



Otter trawl

PLATE III

b To enable them to bite into hard bottoms, or to be used in strong currents, weights may be attached to bring the total weight up to between 22.7 and 45.4 kg.

c Areas sampled range from 1/5th to 1/20th square meters.

d A Petersen grab to be hauled by hand should be fitted with (15 mm or 20 mm) diameter twisted rope in order to provide adequate hand grip. It is best handled by means of wire ropes and a winch.

3 Other bottom samplers include the VanVeen, Lee, Holme, Smith-McIntyre, Knudsen, Ponar, KAJAK and others.

4 A spring loaded sampler was developed by Shipek for use on all types of bottoms. It takes a half-cylinder sample, 1/25th square meters in area and approximately 10 cm deep at the center. The device is automatically triggered on contact with the bottom, and the sample is completely protected enroute to the surface. (Plate I)

5 Drag dredges or scrapes are often used in marine waters and deeper lakes and streams, and comprise the basic equipment of several types of commercial fisheries. Some types have been developed for shallow streams. In general however, they have been little used in fresh water.

6 The above is only a partial listing of the many sampling devices available. Others that are often encountered are the orange-peel bucket, plow dredge, scallop type dredge, hydraulic dredges, and various coring devices. Each has its own advantages and disadvantages and it is up to the worker and his operation to decide what is best for his particular needs. The Petersen and Ekman grabs and and corers are perhaps the most commonly used.

7 Traps of many types are used for various benthic organisms, especially crabs and lobsters. Artificial substrates (below) are in essence a type of trap.

8 Non-randon distribution of biological communities is a real challenge for the biologist.

E Manipulated substrates rely on the ecological predilection of organisms to settle wherever they find a suitable habitat. When a artificial habitat is provided, it tends to become populated by all available species partial to that type of situation. The collector can then at will remove the habitat or trap to his laboratory and study the population at leisure. Two weeks for periphyton and four weeks exposure time for macroinvertebrates are the usual standardized time frames.

This versatile research technique is much used for both routine monitoring and exploratory studies of pollution. It is also exploited commercially, especially for shellfish production. Types of materials use include:

1 Cement plates and panels.

2 Wood (especially for burrowing forms).

3 Glass slides (diatometer or periphytometer).

4 Hester-Dendy multiple plate trap (masonite).

5 Baskets (or other containers) holding natural bottom material and either imbedded in the bottom, or suspended in the overlying water.

6 Unadorned ropes suspended in the water, or sticks thrust into the bottom.

7 A variety of plastic forms.

F Sorting and Preservation of Collections

1 Benthic collections usually consist of a great mass of mud and other debris among which the organisms are hidden. Various procedures may be followed to separate the organisms.

a The organisms may be picked out on the spot by hand or the entire mess taken into the laboratory where it can be examined more efficiently (especially in rough weather). Roughly equivalent time will probably be required in either case.

b Specimens may be observed and recorded or preserved as a permanent record.

c Organisms may be simply counted, weighed, or measured volumetrically; or they may be separated and recorded in groups or species.

If separation is in the field this is usually done by hand picking, screening, or some type of flotation process. The less debris or substrate in the sample reduces picking time.

a Hand picking is best done on a white enameled tray using light touch limnological forceps.

b Screening is one of the most practical methods to separate organisms from debris in the field. Some prefer to use a single fine screen, others prefer a series of 2 or 3 screens of graded sizes. The collection may be dumped directly on the screen and the mud and debris washed through, or it may be dumped into a bucket or small tub. Water is then added, the mixture is well stirred, and the supernatant poured through the partially submerged screen. While doing this the screen is gently agitated or swirled. The residue is then examined for heavy forms that will not float up.

c A variation of this method in situations where there is no mud is to pour a strong sugar or salt solution over the collection in the bucket, stir it well, and again pour the supernatant through the screen. This time, however, saving the flotation solution for re-use. The heavier-than-water solution accentuates the separation of organisms from the debris (except for the heavy shelled molluscs, etc.). A solution of 1.13 kg. of sugar per 3.79L of water is considered to be optimum.

3 Preservation or stabilization is usually necessary in the field. Rose bengal may be added to the preservation to aid picking by staining the organisms.

a 95% ethanol (ethyl alcohol) is highly satisfactory. A final strength of 70% is necessary for prolonged storage. If the collection is drained of water and flooded with 95% ethanol in the field, a laboratory flotation separation can usually be made later, thus saving much time. Considerable quantities of ethanol are required for this procedure.

b Formaldehyde is more widely available and is effective in concentrations of 3 - 10% of the commercial formulation. However, it is highly suspect and is not recommended.

c Properly preserved benthos samples may be retained indefinitely, thereby enhancing their utility and ultimate value.

d Refrigeration or icing is very helpful if many collections made in a short time are to be picked by few individuals.

e There is no single all purpose preservative for unsorted samples. For example some specialists would like their material killed in boiling water. fixed in formalin or other solution and preserved in alcohol. The field investigator usually compromises because of this problem. Again it all depends on the study objectives.

V MICROFAUNA AND PERIPHYTON (OR AUFWUCHS) SAMPLING

A This is a relatively new area which promises to be of great importance. The microfauna of mud and sand bottoms may be studied to some extent from collections made with the various devices mentioned above. In most cases however, there is considerable loss of the smaller forms.

B Most special microfauna samplers for soft bottoms are essentially modified core samplers in which an effort is made to bring up an undisturbed portion of the bottom along with the immediately overlying water. The best type currently seems to be the Enequist sampler which weighs some 35 kg. and takes a 100 sq. cm sample 50 cm. deep.

C Microfauna from the surface of hard sand or gravel bottoms may be sampled by the Hunt vacuum sampler. This has a bell-shaped "sampling" tube sealed by glass diaphragm. On contact with the bottom, the glass is automatically broken and the nearly bottom material is swept up into a trap.

D Periphyton attached to or associated with hard surfaces such as rock or wood may be sampled by scraping or otherwise removing all surface material from a measured area. The periphyton, however, is more effectively quantitatively sampled by manipulated substrate techniques described above.

VI THE COLLECTION, OR SAMPLING OF PLANKTON PLATE IV

A Phytoplankton: A Planned Program is Desirable

1 A planned program of plankton analysis should involve periodic sampling at weekly or even more frequent intervals.

2 A well-planned study or analysis of the growth pattern of plankton in one year will provide a basis for predicting conditions the following year since seasonal growth patterns tend to repeat themselves from year to year.

a Since the seasons and the years differ, records accumulated over the years become more useful.

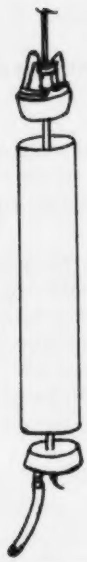
b As the time for an anticipated bloom of some troublesome species approaches, the frequency of analyses may be increased.

3 Detection of a bloom in its early stages will facilitate more economical control.

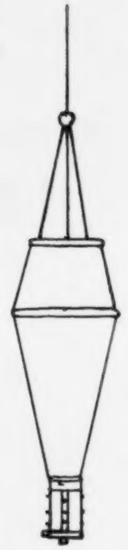
B Field Aspects of the Analysis Program

1 Two general aspects of plankton analysis are commonly recognized: quantitative and qualitative.

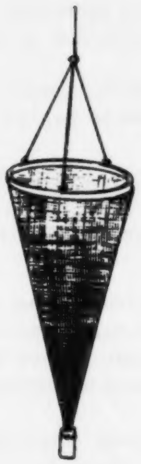
PLANKTON SAMPLERS

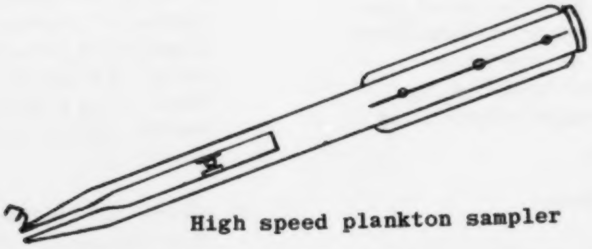


Kemmerer



Wisconsin net





High speed plankton sampler

PLATE IV

a Qualitative examination tells what is present.

b Quantitative tells how much.

c Either approach is useful, a combination is best.

2 Equipment for collecting samples in the field is varied.

a A half-liter bottle will serve for surface samples of phytoplankton, if carefully taken.

b A Kemmerer, Nansen, or other special sampler (small battery operated pumps are time saving) is suggested for depth samples.

c Plankton nets concentrate the sample in the act of collecting and also capture certain larger forms which escape from the bottles. Only the more elaborate types are quantitative however. For phytoplankton, #20 or #25 size nets are commonly used. Usually a net diameter of 13-26 cm is sufficient. Smaller forms, will pass any net.

d The dilemma in choosing a net lies in the fact that larger meshes allow wanted forms to escape while smaller mesh nets tend to clog and increase evasion possibilities of wanted forms.

C Zooplankton Collecting

1 Since zooplankton have the ability to evade sampling devices, nets towed at moderately fast speed are used for their capture. Number 12 nets (aperature size 0.119 mm, 125 meshes 1 inch) or smaller numbered net sizes are commonly used. A net diameter greater than 13 cm is preferred.

Frequently half meter nets or larger are employed. These may be equipped with flow measuring devices for measuring the amount of water entering the net.

3 The devices used for collecting plankton capture both the plant and animal types. The mesh size (net no.) is a method for selecting which category of plankton is to be collected.

D The Location of Sampling Points

1 Both shallow and deep samples are suggested.

a "Shallow" samples should be taken at a depth of 15 cm to 30 cm. The surface film is often significant.

b "Deep" samples should be taken such intervals between surface and bottom as circumstances dictate. In general, the entire water column should be sampled as completely as practicable, and the plankton from each level recorded separately.

2 For estuarine plankton, it is necessary to sample different periods in the stage of the tide, otherwise samples would be biased to a given time, or type of water carried by the tidal currents.

3 Plankton is subjected to the force of the winds and currents. As a result, the plankton is often in patches or "wind rows" (Langmuir cells). For this reason when using a net, it is often desirable to tow the net at right angles to the wind or current.

4 Nearly all plankton are horizontally discontinuous. Planktonic organisms tend to be numerous near the bottom in daylight, but distributed more evenly through the water column at night. Therefore, a series of tows or samples at different depths is necessary to obtain a complete sampling. One technique often employed is to take an oblique tow from the bottom to the top of the water column.

5 Pilot studies to indicate sampling locations and intervals are often mandatory. Some studies require random sampling points.

6 The number of sampling stations that should be established is limited by the capability of the laboratory to analyze the samples, but should approach the needs of the objectives as closely as possible.

7 Field conditions greatly affect the plankton, and a record thereof should be carefully identified with the collection as in II above.

8 Provisions should be made for the field stabilization of the sample until the laboratory examination can be made if more than an hour or so is to elapse.

a Refrigeration or icing is very helpful, but ice should never be placed in the sample.

b Lugols solution is a good preservative.

c Ultra-violet sterilization is sometimes used in the laboratory to retard the decomposition of plankton.

d A highly satisfactory merthiolate preservative has been described by Weber (1968).

VII COLLECTING FISH AND OTHER NEKTON PLATES V, VI

A Fish and other nekton must be sought in the obscure and unlikely areas as well as the obvious locations in order for the collection to be complete. Several techniques should be employed whereever possible (this is appropriate for all biota). It is advisable to check with local authorities to inform them of the reasons for sampling, because many of the techniques are not legal for the layman. In this area, perhaps more than any other, professionally trained workers are important. Also, there must be at least one helper, as a single individual always has difficulty in pulling both ends of a 7 meter seine simultaneously!

The more common techniques are listed below.

B Seines

1 Straight seines range from 2 meters and upwards in length. "Common sense" minnow seines with approximately 6 mm mesh are widely used along shore for collecting the smaller fishes.

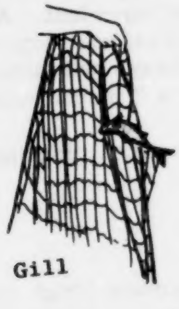
2 Bag seines have an extra trap or bag tied in the middle which helps trap and hold fish when seining in difficult situations.

C Gill nets are of use in offshore and/or deep waters. They range in length from approximately 27.4 meters upward. A mesh size is designed to catch a specified size of fish. The trammel net is a variation of the gill net.

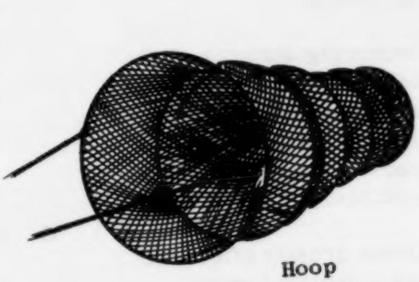
D Traps range from small wire boxes or cylinders with inverted cone entrances to semi-permanent weirs a half mile or more in length. All tend to induce fish to swim into an inner chamber protected by an inverted cone or V - shaped notch to prevent escape. Current operated rotating fish traps are also very effective (and equally illegal) in suitable situations.

E Trawls are submarine nets, usually of considerable size, towed by vessels at speeds sufficient to overtake and scoop in fish, etc. The mouth of the net must be held open by some device such as a long beam (beam trawl) or two or more vanes or "otter boards" (otter trawl). Plate III

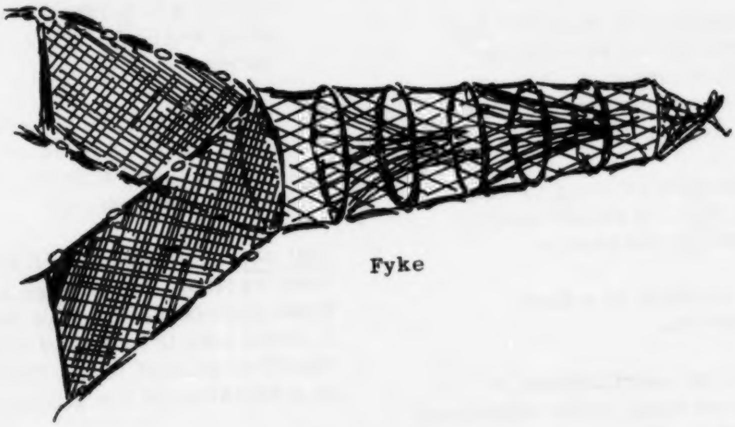
FISH NETS



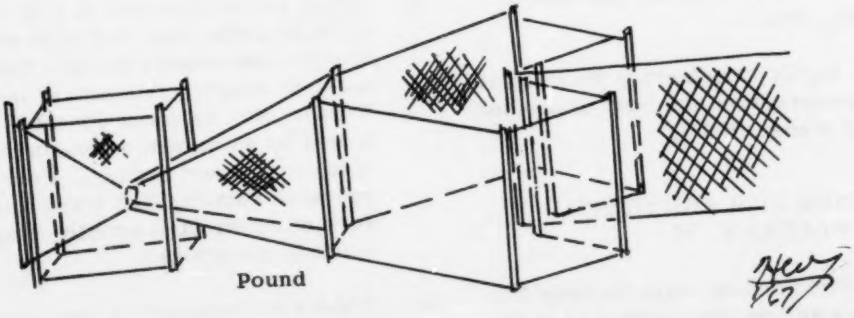
Gill



Hoop

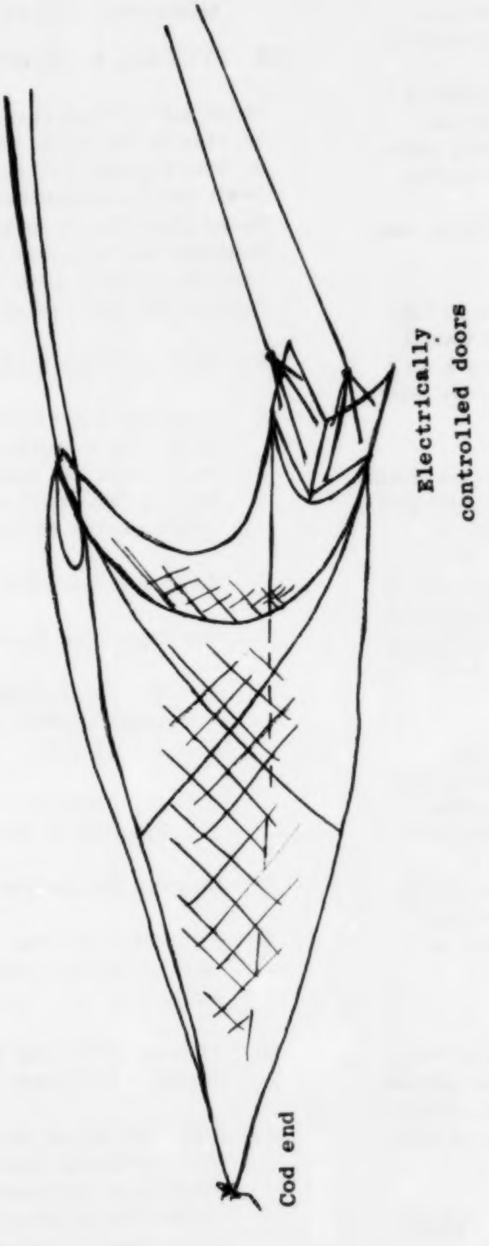


Fyke



Pound

PLATE V


Electrically controlled doors
Cod end

MIDWATER TRAWL

PLATE VI

PLATE VI

1 Beam and otter trawls are usually fished on the bottom, but otter trawls when suitably rigged are now being used to fish mid-depths.

2 The midwater trawl resembles a huge plankton net many feet in diameter. It is proving very effective for collecting at mid-depths.

Numerous special designs have been developed. Plate VI

F Electric seines and screens are widely employed by fishery workers in small and difficult streams. They may also be used in shallow water like areas with certain reservations.

G Poisoning is much used in fishery studies and management. Most widely used and generally satisfactory is rotenone in varying formulations, although many others have been employed from time to time, and some appear to be very good. Under suitable circumstances, fish may even be killed selectively according to species.

H Personal observation by competent personnel, and also informal inquiries and discussions with local residents will often yield information of real use. Many laymen are keen observers, although they do not always understand what they are seeing. The organized creel census technique yields data on what and how many fish are being caught.

I Angling remains in its own right a very good technique in the hands of the skilled practitioner, for determining what fish are present. Spear-fishing also is now being used in some studies.

J Fish and other nekton are often tagged to trace their movements during migration and at other times. Miniature radio transmitters can now be attached or fed to fish (and other organisms) which enable them to be tracked over considerable distances. Physiological information is often obtained in this way. This is known as telemetry (remote sensing).

VIII SPECIAL REQUIREMENTS ON BOATS

Handling biological collections (as contrasted to chemical and physical sampling) on board boats differs with the size of the craft and the magnitude of operations. Some possible items are listed below. Hoisting and many other types of gear are used in common with other types of collection, and will not be listed.

A Special Laboratory Room(s)

B Constant flow of Clean water for culturing organisms. (Selection of materials and design of a system to insure non-toxic water may be very troublesome but very important.)

C Live Box built into ship at water level

D Refrigeration System(s)

1 For controlling temperature of experimental organisms in laboratory.

2 For deep-freezing and storage of specimens to be examined later.

E Storage Space (Unrefrigerated)

F Facilities for the safe storage and use of microscopes and other laboratory equipment.

G Facilities for the safe storage and use of deck equipment.

H Administrative access to the Captain and Technical Leader in order to coordinate requirements for biological collection (such as a slow plankton tow) with those for other collections.

I Safety of personnel working in and around boats, as well as in other field activities should be seriously considered and promoted at all times.

IX OTHER TYPES OF BIOLOGICAL FIELD STUDIES INCLUDE

A Productivity Studies of Many Types

B Life Cycle and Management

C Distribution of Sport or (potentially) Commercial Species

D Scattering Layers and Other Submarine Sound Studies

E Artificial Culture of Marine Food Crops

F Radioactive Uptake

G Growth of Surface-Fouling Organisms

H Marine Borers

I Dangerous Marine Organisms

J Red Tides

K Others

X SOURCES OF COLLECTING EQUIPMENT

Many specialized items of biological collecting equipment are not available from the usual laboratory supply houses. Consequently, the American Society of Limnology and Oceanography has compiled a list of companies handling such items and released it as "Special Publication No. 1, Sources of Limnoligical and Oceanographic Apparatus and Supplies." Available from the Secretary of the Society.

XI SAFETY

The hazards associated with work on or near water require special consideration. Personnel should not be assigned to duty alone in boats, and should be competent in the use of boating equipment (courses are offered by the U. S. Coast Guard). Field training should also include instructions on the proper rigging and handling of biological sampling gear.

Life preservers(jacket type work vests) should be worn at all times when on or near deep water. Boats should have air-tight or foam-filled compartments for flotation and be equipped with fire extinguishers, running lights, oars, and anchor. Coast Guard regulations should always be observed. All boat trailers should have two rear running and stop lights and turn signals and a license plate illuminator. Trailers 2 meters (wheel to wheel) or more wide should be equipped with amber marker lights on the front and rear of the frame on both sides.

Laboratories should be provided with fire extinguishers, fume hoods, and eye fountains. Safety glasses should be worn when mixing dangerous chemicals and preservatives.

XII BIOLOGICAL METHODS

Biological methods and samples are in many ways analagous to chemical methods and samples. They are unique however in many other ways especially so because of inherent biological variability.

A The organisms are nearly always distributed non-randomly.

B There is much seasonal variation.

C There is much diel periodicity (24 hour activity periods).

D Properly handled and preserved, they may retain their integrity indefinitely.

E Most biological samples require a biologist to collect.

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This outline was prepared by H.W. Jackson, Former Chief Biologist, National Training and Operational Technology Center, OWPO, EPA, Cincinnati, OH 45268, and revised by R. M. Sinclair, Aquatic Biologist, National Training and Operational Technology Center.

Descriptors:
Aquatic Environment, Analytical Techniques, Sampling, On-Site Investigations, Preservation, Samplers, Water Sampling, Handling, Sample, Surface Waters, Aquatic Life, Biological Methods

THE AQUATIC ENVIRONMENT

Part 1: The Nature and Behavior of Water

I INTRODUCTION

The earth is physically divisible into the lithosphere or land masses, and the hydrosphere which includes the oceans, lakes, streams, and subterranean waters; and the atmosphere.

A Upon the hydrospere are based a number of sciences which represent different approaches. Hydrology is the general science of water itself with its various special fields such as hydrography, hydraulics, etc. These in turn merge into physical chemistry and chemistry.

B Limnology and oceanography combine aspects of all of these, and deal not only with the physical liquid water and its various naturally occurring solutions and forms, but also with living organisms and the infinite interactions that occur between them and their environment.

C Water quality management, including pollution control, thus looks to all branches of aquatic science in efforts to coordinate and improve man's relationship with his aquatic environment.

II SOME FACTS ABOUT WATER

A Water is the only abundant liquid on our planet. It has many properties most unusual for liquids, upon which depend most of the familiar aspects of the world about us as we know it. (See Table 1)

TABLE 1

UNIQUE PROPERTIES OF WATER

Property	Significance
Highest heat capacity (specific heat) of any solid or liquid (except NH_3)	Stabilizes temperatures of organisms and geographical regions
Highest latent heat of fusion (except NH_3)	Thermostatic effect at freezing point
Highest heat of evaporation of any substance	Important in heat and water transfer of atmosphere
The only substance that has its maximum density as a liquid (4° C)	Fresh and brackish waters have maximum density above freezing point. This is important in vertical circulation pattern in lakes.
Highest surface tension of any liquid	Controls surface and drop phenomena, important in cellular physiology
Dissolves more substances in greater quantity than any other liquid	Makes complex biological system possible. Important for transportation of materials in solution.
Pure water has the highest di-electric constant of any liquid	Leads to high dissociation of inorganic substances in solution
Very little electrolytic dissociation	Neutral, yet contains both H^+ and OH^- ions
Relatively transparent	Absorbs much energy in infra red and ultra violet ranges, but little in visible range. Hence "colorless"

B Physical Factors of Significance

1 Water substance

Water is not simply "H_2O" but in reality is a mixture of some 33 different substances involving three isotopes each of hydrogen and oxygen (ordinary hydrogen H^1, deuterium H^2, and tritium H^3; ordinary oxygen O^{16}, oxygen 17, and oxygen 18) plus 15 known types of ions. The molecules of a water mass tend to associate themselves as polymers rather than to remain as discrete units. (See Figure 1)

SUBSTANCE OF PURE WATER

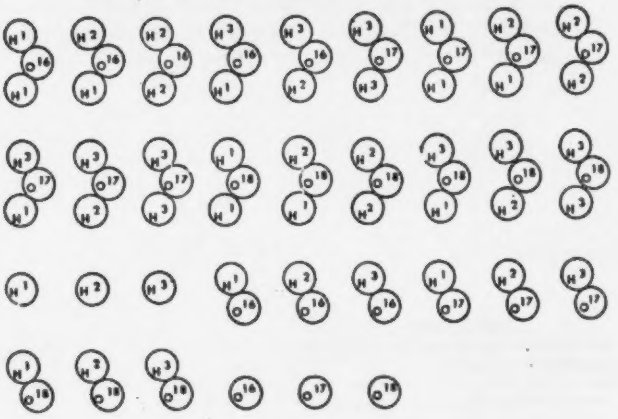
H1 O16 H1
H2 O16 H1
H2 O16 H2
H3 O16 H1
H3 O16 H2
H3 O17 H3
H1 O17 H1
H2 O17 H1
H2 O17 H2
H3 O17 H1
H3 O17 H2
H3 O17 H3
H1 O18 H1
H2 O18 H1
H2 O18 H2
H3 O18 H1
H3 O18 H2
H3 O18 H3
H1
H2
H3
H1 O16
H2 O16
H3 O16
H1 O17
H2 O17
H3 O17
H1 O18
H2 O18
H3 O18
O16
O17
O18

Figure 1

2 Density

a Temperature and density: Ice. Water is the only known substance in which the solid state will float on the liquid state. (See Table 2)

TABLE 2

EFFECTS OF TEMPERATURE ON DENSITY OF PURE WATER AND ICE*

Temperature (°C)	Density	
	Water	Ice**
-10	.99815	.9397
- 8	.99869	.9360
- 6	.99912	.9020
- 4	.99945	.9277
- 2	.99970	.9229
0	.99987----	.9168
2	.99997	
4	1.00000	
6	.99997	
8	.99988	
10	.99973	
20	.99823	
40	.99225	
60	.98324	
80	.97183	
100	.95838	

* Tabular values for density, etc., represent estimates by various workers rather than absolute values, due to the variability of water.

** Regular ice is known as "ice I". Four or more other "forms" of ice are known to exist (ice II, ice III, etc.), having densities at 1 atm. pressure ranging from 1.1595 to 1.67. These are of extremely restricted occurrence and may be ignored in most routine operations.

This ensures that ice usually forms on top of a body of water and tends to insulate the remaining water mass from further loss of heat. Did ice sink, there could be little or no carryover of aquatic life from season to season in the higher latitudes. Frazil or needle ice forms colloidally at a few thousandths of a degree below 0° C. It is adhesive and may build up on submerged objects as "anchor ice", but it is still typical ice (ice I).

1) Seasonal increase in solar radiation annually warms surface waters in summer while other factors result in winter cooling. The density differences resulting establish two classic layers: the epilimnion or surface layer, and the hypolimnion or lower layer, and in between is the thermocline or shear-plane.

2) While for certain theoretical purposes a "thermocline" is defined as a zone in which the temperature changes one degree centigrade for each meter of depth, in practice, any transitional layer between two relatively stable masses of water of different temperatures may be regarded as a thermocline.

3) Obviously the greater the temperature differences between epilimnion and hypolimnion and the sharper the gradient in the thermocline, the more stable will the situation be.

4) From information given above, it should be evident that while the temperature of the hypolimnion rarely drops much below 4° C, the epilimnion may range from 0° C upward.

5) When epilimnion and hypolimnion achieve the same temperature, stratification no longer exists. The entire body of water behaves hydrologically as a unit, and tends to assume uniform chemical and physical characteristics. Even a light breeze may then cause the entire body of water to circulate. Such events are called overturns, and usually result in water quality changes of considerable physical, chemical, and biological significance. Mineral-rich water from the hypolimnion, for example, is mixed with oxygenated water from the epilimnion. This usually triggers a sudden growth or "bloom" of plankton organisms.

6) When stratification is present, however, each layer behaves relatively independently, and significant quality differences may develop.

7) Thermal stratification as described above has no reference to the size of the water mass; it is found in oceans and puddles.

b The relative densities of the various isotopes of water influence its molecular composition. For example, the lighter O_{16} tends to go off first in the process of evaporation, leading to the relative enrichment of air by O_{16} and the enrichment of water by O_{17} and O_{18}. This can lead to a measurably higher O_{18} content in warmer climates. Also, the temperature of water in past geologic ages can be closely estimated from the ratio of O_{18} in the carbonate of mollusc shells.

c Dissolved and/or suspended solids may also affect the density of natural water masses (see Table 3)

TABLE 3
EFFECTS OF DISSOLVED SOLIDS ON DENSITY

Dissolved Solids (Grams per liter)	Density (at 4° C)
0	1.00000
1	1.00085
2	1.00169
3	1.00251
10	1.00818
35 (mean for sea water)	1.02822

d Types of density stratification

1) Density differences produce stratification which may be permanent, transient, or seasonal.

2) Permanent stratification exists for example where there is a heavy mass of brine in the deeper areas of a basin which does not respond to seasonal or other changing conditions.

3) Transient stratification may occur with the recurrent influx of tidal water in an estuary for example, or the occasional influx of cold muddy water into a deep lake or reservoir.

4) Seasonal stratification is typically thermal in nature, and involves the annual establishment of the epilimnion, hypolimnion, and thermocline as described above.

5) Density stratification is not limited to two-layered systems; three, four, or even more layers may be encountered in larger bodies of water.

e A "plunge line" (sometimes called "thermal line") may develop at the mouth of a stream. Heavier water flowing into a lake or reservoir plunges below the lighter water mass of the epiliminium to flow along at a lower level. Such a line is usually marked by an accumulation of floating debris.

f Stratification may be modified or entirely suppressed in some cases when deemed expedient, by means of a simple air lift.

3 The viscosity of water is greater at lower temperatures (see Table 4).

This is important not only in situations involving the control of flowing water as in a sand filter, but also since overcoming resistance to flow generates heat, it is significant in the heating of water by internal friction from wave and current action. Living organisms more easily support themselves in the more viscous (and also denser) cold waters of the arctic than in the less viscous warm waters of the tropics. (See Table 4).

TABLE 4

VISCOSITY OF WATER (In millipoises at 1 atm)

	Dissolved solids in g/L			
Temp. °C	0	5	10	30
-10	26.0	----	----	----
- 5	21.4	----	----	----
0	17.94	18.1	18.24	18.7
5	15.19	15.3	15.5	16.0
10	13.10	13.2	13.4	13.8
30	8.00	8.1	8.2	8.6
100	2.84	----	----	----

4 Surface tension has biological as well as physical significance. Organisms whose body surfaces cannot be wet by water can either ride on the surface film or in some instances may be "trapped" on the surface film and be unable to re-enter the water.

5 Heat or energy

Incident solar radiation is the prime source of energy for virtually all organic and most inorganic processes on earth. For the earth as a whole, the total amount (of energy) received annually must exactly balance that lost by reflection and radiation into space if climatic and related conditions are to remain relatively constant over geologic time.

a For a given body of water, immediate sources of energy include in addition to solar irradiation: terrestrial heat, transformation of kinetic energy (wave and current action) to heat, chemical and biochemical reactions, convection from the atmosphere, and condensation of water vapor.

b The proportion of light reflected depends on the angle of incidence, the temperature, color, and other qualities of the water; and the presence or absence of films of lighter liquids such as oil. In general, as the depth increases arithmetically, the light tends to decrease geometrically. Blues, greens, and yellows tend to penetrate most deeply while ultra violet, violets, and orange-reds are most quickly absorbed. On the order of 90% of the total illumination which penetrates the surface film is absorbed in the first 10 meters of even the clearest water, thus tending to warm the upper layers.

6 Water movements

a Waves or rhythmic movement

1) The best known are traveling waves caused by wind. These are effective only against objects near the surface. They have little effect on the movement of large masses of water.

2) Seiches

Standing waves or seiches occur in lakes, estuaries, and other enclosed bodies of water, but are seldom large enough to be observed. An "internal wave or seich" is an oscillation in a submersed mass of water such as a hypolimnion, accompanied by compensating oscillation in the overlying water so that no significant change in surface level is detected. Shifts in submerged water masses of this type can have severe effects on the biota and also on human water uses where withdrawals are confined to a given depth. Descriptions and analyses of many other types and sub-types of waves and wave-like movements may be found in the literature.

b Tides

1) Tides are the longest waves known, and are responsible for the once or twice a day rythmic rise and fall of the ocean level on most shores around the world.

2) While part and parcel of the same phenomenon, it is often convenient to refer to the rise and fall of the water level as "tide," and to the resulting currents as "tidal currents."

3) Tides are basically caused by the attraction of the sun and moon on water masses, large and small; however, it is only in the oceans and possibly certain of the larger lakes that true tidal action has been demonstrated. The patterns of tidal action are enormously complicated by local topography, interaction with seiches, and other factors. The literature on tides is very large.

c Currents (except tidal currents) are steady arythmic water movements which have had major study only in oceanography although they are most often observed in rivers and streams. They are primarily concerned with the translocation of water masses. They may be generated internally by virtue of density changes, or externally by wind or terrestrial topography. Turbulence phenomena or eddy currents are largely responsible for lateral mixing in a current. These are of far more importance in the economy of a body of water than mere laminar flow.

d Coriolis force is a result of interaction between the rotation of the earth, and the movement of masses or bodies on the earth. The net result is a slight tendency for moving objects to veer to the right in the northern hemisphere, and to the left in the southern hemisphere. While the result in fresh waters is usually negligible, it may be considerable in marine waters. For example, other factors permitting, there is a tendency in estuaries for fresh waters to move toward the ocean faster along the right bank, while salt tidal waters tend to intrude farther inland along the left bank. Effects are even more dramatic in the open oceans.

e Langmuire spirals (or Langmuire circulation) are a relatively massive cylindrical motion imparted to surface waters under the influence of wind. The axes of the cylinders are parallel to the direction of the wind, and their depth and velocity depend on the depth of the water, the velocity and duration of the wind, and other factors. The net result is that adjacent cylinders tend to rotate in opposite directions like meshing cog wheels. Thus the water between two given spirals may be meeting and sinking, while that between spirals on either side will be meeting and rising. Water over the sinking, while that between spirals on either side will be meeting and rising. Water over the sinking areas tends to accumulate flotsam and jetsam on the surface in long conspicuous lines.

a This phenomenon is of considerable importance to those sampling for plankton (or even chemicals) near the surface when the wind is blowing. Grab samples from either dance might obviously differ considerably, and if a plankton tow is contemplated it should be made across the wind in order that the net may pass through a succession of both dances.

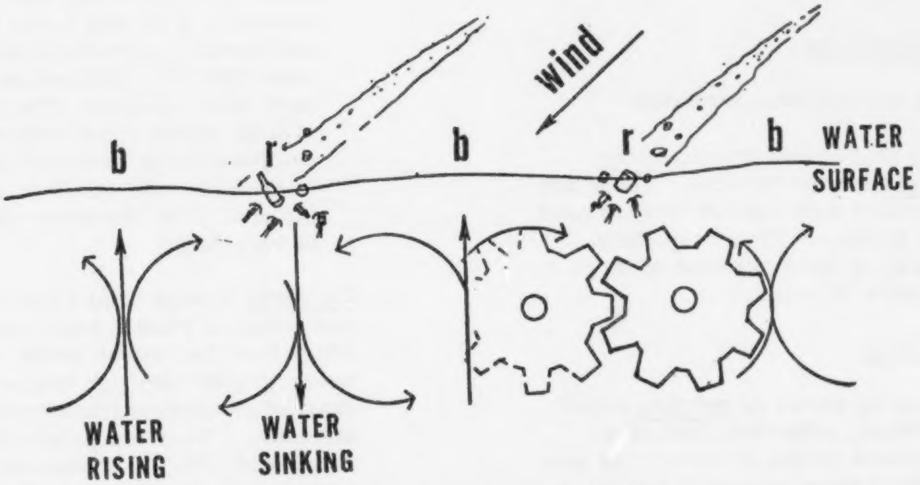

Figure 2. Langmuire Spirals
b. Blue dance, water rising. r. Red dance, water sinking, floating or swimming objects concentrated.

b Langmuire spirals are not usually established until the wind has either been blowing for an extended period, or else is blowing rather hard. Their presence can be detected by the lines of foam and other floating material which coincide with the direction of the wind.

6 The pH of pure water has been determined between 5.7 and 7.01 by various workers. The latter value is most widely accepted at the present time. Natural waters of course vary widely according to circumstances.

C The elements of hydrology mentioned above represent a selection of some of the more conspicuous physical factors involved in working with water quality. Other items not specifically mentioned include: molecular structure of waters, interaction of water and radiation, internal pressure, acoustical characteristics, pressure-volume-temperature relationships, refractivity, luminescence, color, dielectrical characteristics and phenomena, solubility, action and interactions of gases, liquids and solids, water vapor, phenomena of hydrostatics and hydrodynamics in general.

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Part 2: The Aquatic Environment as an Ecosystem

I INTRODUCTION

Part 1 introduced the lithosphere and the hydrosphere. Part 2 will deal with certain general aspects of the biosphere, or the sphere of life on this earth, which photographs from space have shown is a finite globe in infinite space.

This is the habitat of man and the other organisms. His relationships with the aquatic biosphere are our common concern.

II THE BIOLOGICAL NATURE OF THE WORLD WE LIVE IN

A We can only imagine what this world must have been like before there was life.

B The world as we know it is largely shaped by the forces of life.

1 Primitive forms of life created organic matter and established soil.

2 Plants cover the lands and enormously influence the forces of erosion.

3 The nature and rate of erosion affect the redistribution of materials (and mass) on the surface of the earth (topographic changes).

4 Organisms tie up vast quantities of certain chemicals, such as carbon and oxygen.

5 Respiration of plants and animals releases carbon dioxide to the atmosphere in influential quantities.

6 CO_2 affects the heat transmission of the atmosphere.

C Organisms respond to and in turn affect their environment. Man is one of the most influential.

III ECOLOGY IS THE STUDY OF THE INTERRELATIONSHIPS BETWEEN ORGANISMS, AND BETWEEN ORGANISMS AND THEIR ENVIRONMENT.

A The ecosystem is the basic functional unit of ecology. Any area of nature that includes living organisms and nonliving substances interacting to produce an exchange of materials between the living and nonliving parts constitutes an ecosystem. (Odum, 1959)

1 From a structural standpoint, it is convenient to recognize four constituents as composing an ecosystem (Figure 1).

a Abiotic NUTRIENT MINERALS which are the physical stuff of which living protoplasm will be synthesized.

b Autotrophic (self-nourishing) or PRODUCER organisms. These are largely the green plants (holophytes), but other minor groups must also be included (See Figure 2). They assimilate the nutrient minerals, by the use of considerable energy, and combine them into living organic substance.

c Heterotrophic (other-nourishing) CONSUMERS (holozoic), are chiefly the animals. They ingest (or eat) and digest organic matter, releasing considerable energy in the process.

d Heterotrophic REDUCERS are chiefly bacteria and fungi that return complex organic compounds back to the original abiotic mineral condition, thereby releasing the remaining chemical energy.

2 From a functional standpoint, an ecosystem has two parts (Figure 2)

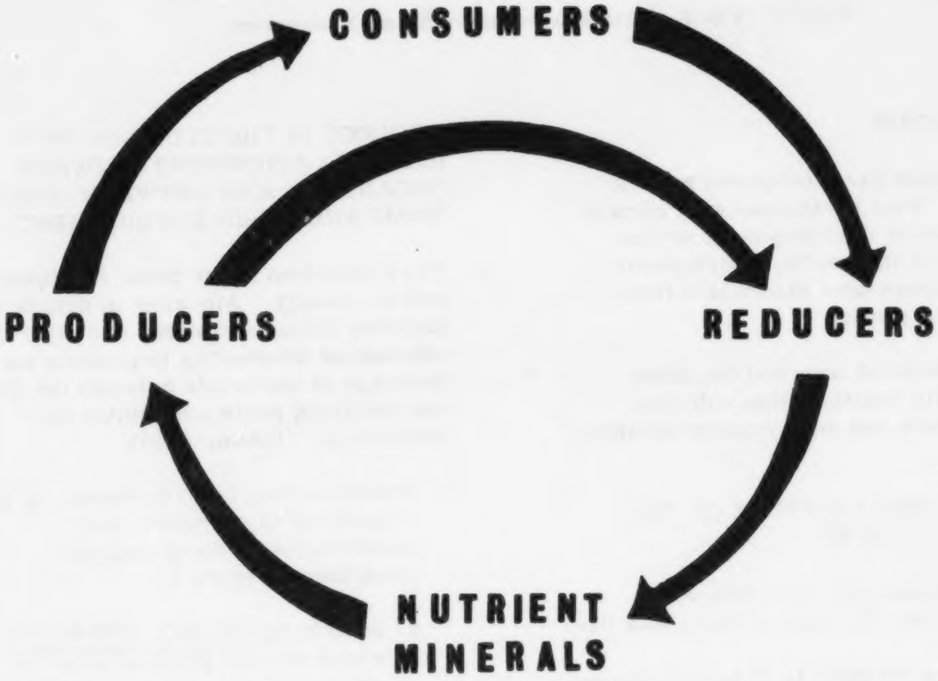

FIGURE 1

a The autotrophic or producter organisms, which utilize light energy or the oxidation of inorganic compounds as their sole energy source.

b The heterotropic or consumer and reducer organisms which utilizes organic compounds for its energy and carbon requirements.

3 Unless the autotrophic and heterotrophic phases of the cycle approximate a dynamic equilibrium, the ecosystem and the environment will change.

B Each of these groups includes simple, single-celled representatives, persisting at lower levels on the evolutionary stems of the higher organisms. (Figure 2)

1 These groups span the gaps between the higher kingdoms with a multitude of transitional forms. They are collectively called the PROTISTA and MONERA.

2 These two groups can be defined on the basis of relative complexity of structure.

a The bacteria and blue-green algae, lacking a nuclear membrane are the Monera.

b The single-celled algae and protozoa are Protista.

C Distributed throughout these groups will be found most of the traditional "phyla" of classic biology.

IV FUNCTIONING OF THE ECOSYSTEM

A A food chain is the transfer of food energy from plants through a series of organisms with repeated eating and being eaten. Food chains are not isolated sequences but are interconnected.

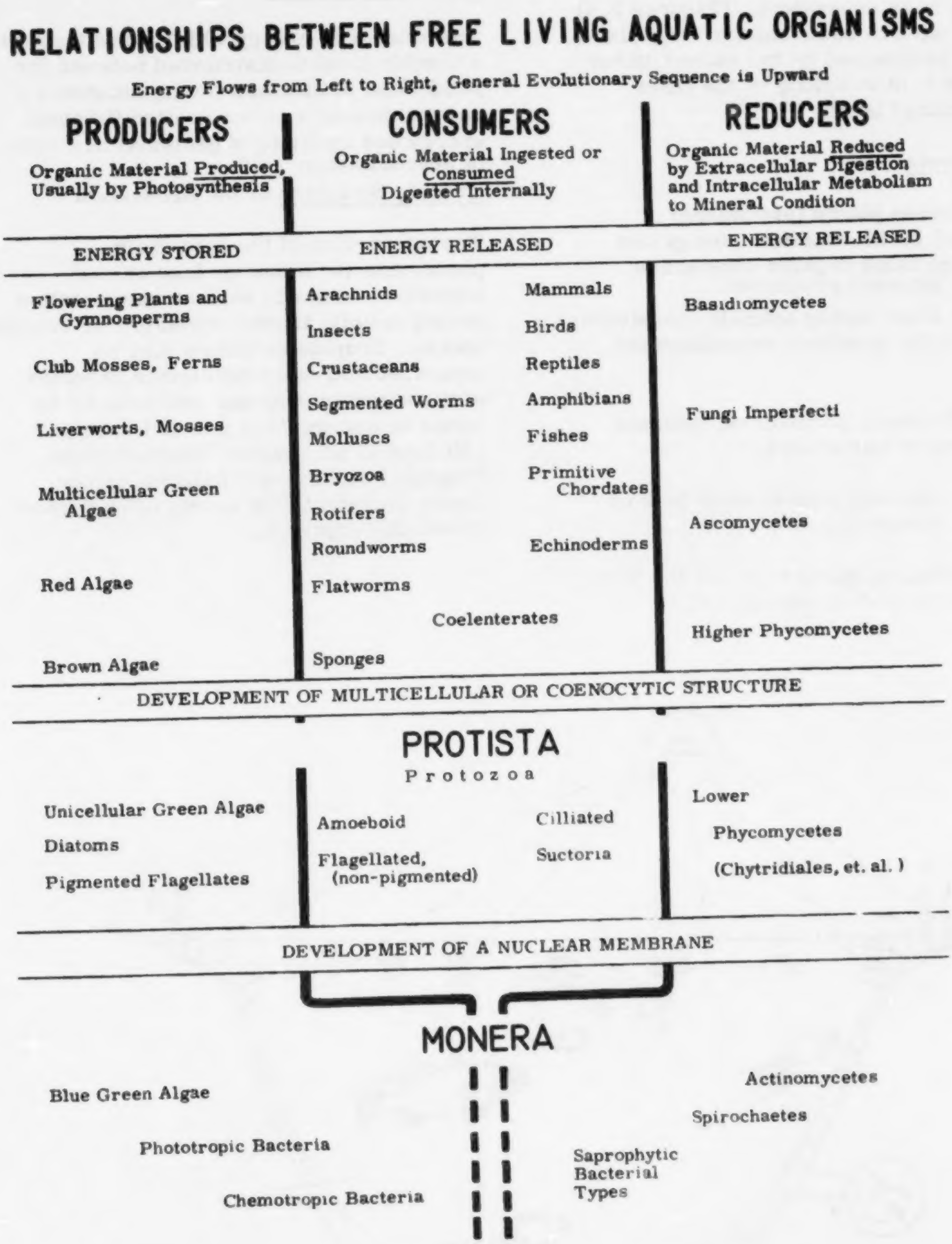
RELATIONSHIPS BETWEEN FREE LIVING AQUATIC ORGANISMS
Energy Flows from Left to Right, General Evolutionary Sequence is Upward
PRODUCERS
Organic Material Produced, Usually by Photosynthesis
CONSUMERS
Organic Material Ingested or Consumed
Digested Internally
REDUCERS
Organic Material Reduced by Extracellular Digestion and Intracellular Metabolism to Mineral Condition
ENERGY STORED
ENERGY RELEASED
ENERGY RELEASED
Flowering Plants and Gymnosperms
Club Mosses, Ferns
Liverworts, Mosses
Multicellular Green Algae
Red Algae
Brown Algae
Arachnids
Insects
Crustaceans
Segmented Worms
Molluscs
Bryozoa
Rotifers
Roundworms
Flatworms
Sponges
Mammals
Birds
Reptiles
Amphibians
Fishes
Primitive Chordates
Echinoderms
Coelenterates
Basidiomycetes
Fungi Imperfecti
Ascomycetes
Higher Phycomycetes
DEVELOPMENT OF MULTICELLULAR OR COENOCYTIC STRUCTURE
PROTISTA
Protozoa
Unicellular Green Algae
Diatoms
Pigmented Flagellates
Amoeboid
Flagellated, (non-pigmented)
Cilliated
Suctoria
Lower Phycomycetes
(Chytridiales, et. al.)
DEVELOPMENT OF A NUCLEAR MEMBRANE
MONERA
Blue Green Algae
Phototropic Bacteria
Chemotropic Bacteria
Saprophytic Bacterial Types
Actinomycetes
Spirochaetes

FIGURE 2

BI. ECO. pl. 2a. 1. 69

B **A food web** is the interlocking pattern of food chains in an ecosystem. (Figures 3, 4) In complex natural communities, organisms whose food is obtained by the same number of steps are said to belong to the same trophic (feeding) level.

C **Trophic Levels**

1 First - Green plants (producers) (Figure 5) fix biochemical energy and synthesize basic organic substances. This is "primary production".

2 Second - Plant eating animals (herbivores) depend on the producer organisms for food.

3 Third - Primary carnivores, animals which feed on herbivores.

4 Fourth - Secondary carnivores feed on primary carnivores.

5 Last - Ultimate carnivores are the last or ultimate level of consumers.

D **Total Assimilation**

The amount of energy which flows through a trophic level is distributed between the production of biomass (living substance), and the demands of respiration (internal energy use by living organisms) in a ratio of approximately 1:10.

E **Trophic Structure** of the Ecosystem

The interaction of the food chain phenomena (with energy loss at each transfer) results in various communities having definite trophic structure or energy levels. Trophic structure may be measured and described either in terms of the standing crop per unit area or in terms of energy fixed per unit area per unit time at successive trophic levels. Trophic structure and function can be shown graphically by means of ecological pyramids (Figure 5).

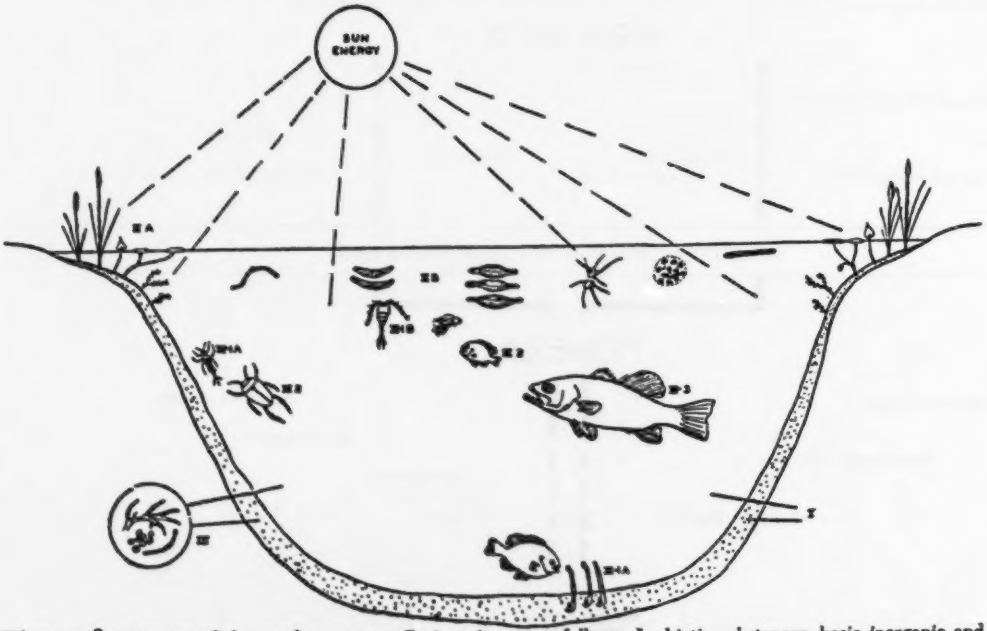

Figure 3. Diagram of the pond ecosystem. Basic units are as follows: I, abiotic substances–basic inorganic and organic compounds; IIA, producers–rooted vegetation; IIB, producers–phytoplankton; III-1A, primary consumers (herbivores)–bottom forms; III-1B, primary consumers (herbivores)–zooplankton; III-2, secondary consumers (carnivores); III-3, tertiary consumers (secondary carnivores); IV, decomposers–bacteria and fungi of decay.

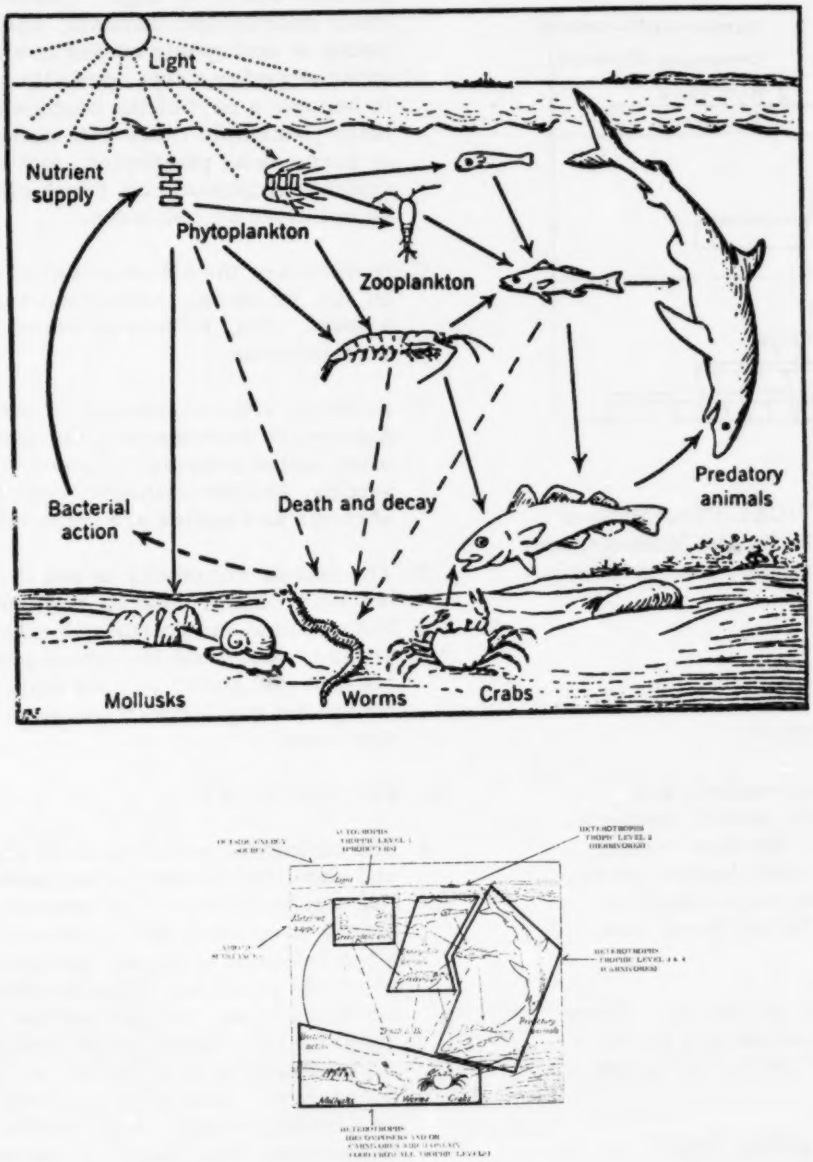
Light
Nutrient supply
Phytoplankton
Zooplankton
Bacterial action
Death and decay
Predatory animals
Mollusks
Worms
Crabs
OUTSIDE ENERGY SOURCE
AUTOTROPHS TROPHIC LEVEL 1 (PRODUCERS)
HETEROTROPHS TROPHIC LEVEL 2 (HERBIVORES)
ABIOTIC SUBSTANCES
HETEROTROPHS TROPHIC LEVEL 3 & 4 (CARNIVORES)
HETEROTROPHS (DECOMPOSERS AND OR CARNIVORES WHICH OBTAIN FOOD FROM ALL TROPHIC LEVELS)

Figure 4. A MARINE ECOSYSTEM (After Clark, 1954 and Patten, 1966)

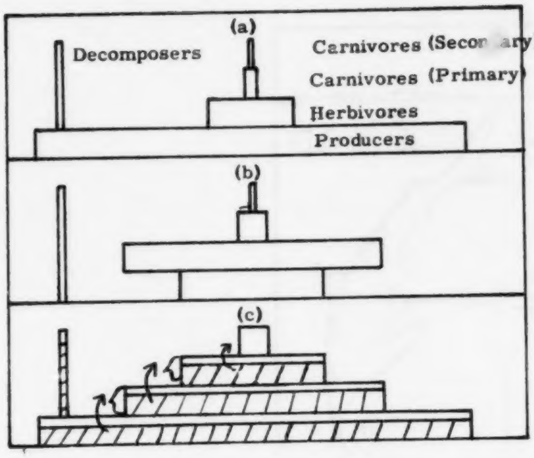

Figure 5. HYPOTHETICAL PYRAMIDS of (a) Numbers of individuals, (b) Biomass, and (c) Energy (Shading Indicates Energy Loss).

V BIOTIC COMMUNITIES

A Plankton are the macroscopic and microscopic animals, plants, bacteria, etc., floating free in the open water. Many clog filters, cause tastes, odors, and other troubles in water supplies. Eggs and larvae of larger forms are often present.

1 Phytoplankton are plant-like. These are the dominant producers of the waters, fresh and salt, "the grass of the seas".

2 Zooplankton are animal-like. Includes many different animal types, range in size from minute protozoa to gigantic marine jellyfishes.

B Periphyton (or Aufwuchs) - The communities of microscopic organisms associated with submerged surfaces of any type or depth. Includes bacteria, algae, protozoa, and other microscopic animals, and often the young or embryonic stages of algae and other organisms that normally grow up to become a part of the benthos (see below). Many planktonic types will also adhere to surfaces as periphyton, and some typical periphyton may break off and be collected as plankters.

C Benthos are the plants and animals living on, in, or closely associated with the bottom. They include plants and invertebrates.

D Nekton are the community of strong aggressive swimmers of the open waters, often called pellagic. Certain fishes, whales, and invertebrates such as shrimps and squids are included here.

E The marsh community is based on larger "higher" plants, floating and emergent. Both marine and freshwater marshes are areas of enormous biological production. Collectively known as "wetlands", they bridge the gap between the waters and the dry lands.

VI PRODUCTIVITY

A The biological resultant of all physical and chemical factors in the quantity of life that may actually be present. The ability to produce this "biomass" is often referred to as the "productivity" of a body of water. This is neither good nor bad per se. A water of low productivity is a "poor" water biologically, and also a relatively "pure" or "clean" water; hence desirable as a water supply or a bathing beach. A productive water on the other hand may be a nuisance to man or highly desirable. It is a nuisance if foul odors and/or weed-chocked waterways result, it is desirable if bumper crops of bass, catfish, or oysters are produced. Open oceans have a low level of productivity in general.

VII PERSISTENT CHEMICALS IN THE ENVIRONMENT

Increasingly complex manufacturing processes, coupled with rising industrialization, create health hazards for humans and aquatic life.

Compounds besides being toxic (acutely or chronic) may produce mutagenic effects including cancer, tumors, and teratogenicity (embryo defects). Fortunately there are tests, such as the Amis test, to screen chemical compounds for these effects.

A Metals - current levels of cadmium, lead and other substances constitute a mounting concern. Mercury pollution, as at Minimata, Japan has been fully documented.

B Pesticides

1 A pesticide and its metabolites may move through an ecosystem in many ways. Hard (pesticides which are persistent, having a long half-life in the environment includes the organochlorines, ex., DDT) pesticides ingested or otherwise borne by the target species will stay in the environment, possibly to be recycled or concentrated further through the natural action of food chains if the species is eaten. Most of the volume of pesticides do not reach their target at all.

2 Biological magnification

Initially, low levels of persistent pesticides in air, soil, and water may be concentrated at every step up the food chain. Minute aquatic organisms and scavengers, which screen water and bottom mud having pesticide levels of a few parts per billion, can accumulate levels measured in parts per million—a thousandfold increase. The sediments including fecal deposits are continuously recycled by the bottom animals.

a Oysters, for instance, will concentrate DDT 70,000 times higher in their tissues than it's concentration in surrounding water. They can also partially cleanse themselves in water free of DDT.

b Fish feeding on lower organisms build up concentrations in their visceral fat which may reach several thousand parts per million and levels in their edible flesh of hundreds of parts per million.

c Larger animals, such as fish-eating gulls and other birds, can further concentrate the chemicals. A survey on organochlorine residues in aquatic birds in the Canadian prairie provinces showed that California and ring-billed gulls were among the most contaminated. Since gulls breed in colonies, breeding population changes can be detected and related to levels of chemical contamination. Ecological research on colonial birds to monitor the effects of chemical pollution on the environment is useful.

C **"Polychlorinated biphenyls" (PCB's). PCB's were used in plasticizers, asphalt, ink, paper, and a host of other products. Action was taken to curtail their release to the environment, since their effects are similar to hard pesticides. However this doesn't solve the problems of contaminated sediments and ecosystems and final fate of the PCB's still circulating.**

D **There are numerous other compounds which are toxic and accumulated in the ecosystem.**

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Part 3. The Freshwater Environment

I INTRODUCTION

The freshwater environment as considered herein refers to those inland waters not detectably diluted by ocean waters, although the lower portions of rivers are subject to certain tidal flow effects.

Certain atypical inland waters such as saline or alkaline lakes, springs, etc., are not treated, as the main objective here is typical inland water.

All waters have certain basic biological cycles and types of interactions most of which have already been presented, hence this outline will concentrate on aspects essentially peculiar to fresh inland waters.

II PRESENT WATER QUALITY AS A FUNCTION OF THE EVOLUTION OF FRESH WATERS

A The history of a body of water determines its present condition. Natural waters have evolved in the course of geologic time into what we know today.

B Streams

In the course of their evolution, streams in general pass through four stages of development which may be called: birth, youth, maturity, and old age.

These terms or conditions may be employed or considered in two contexts: temporal, or spatial. In terms of geologic time, a given point in a stream may pass through each of the stages described below or: at any given time, these various stages of development can be loosely identified in successive reaches of a stream traveling from its headwaters to base level in ocean or major lake.

1 Establishment or birth. This might be a "dry run" or headwater stream-bed, before it had eroded down to the level of ground water. During periods of run-off after a rain or snow-melt, such a gulley would have a flow of water which might range from torrential to a mere trickle. Erosion may proceed rapidly as there is no permanent aquatic flora or fauna to stabilize streambed materials. On the other hand, terrestrial grass or forest growth may retard erosion. When the run-off has passed, however, the "streambed" is dry.

2 Youthful streams. When the streambed is eroded below the ground water level, spring or seepage water enters, and the stream becomes permanent. An aquatic flora and fauna develops and water flows the year round. Youthful streams typically have a relatively steep gradient, rocky beds, with rapids, falls, and small pools.

3 Mature streams. Mature streams have wide valleys, a developed flood plain, are deeper, more turbid, and usually have warmer water, sand, mud, silt, or clay bottom materials which shift with increase in flow. In their more favorable reaches, streams in this condition are at a peak of biological productivity. Gradients are moderate, riffles or rapids are often separated by long pools.

4 In old age, streams have approached geologic base level, usually the ocean. During flood stage they scour their beds and deposit materials on the flood plain which may be very broad and flat. During normal flow the channel is refilled and many shifting bars are developed. Meanders and ox-bow lakes are often formed.

(Under the influence of man this pattern may be broken up, or temporarily interrupted. Thus an essentially "youthful" stream might take on some of the characteristics of a "mature" stream following soil erosion, organic enrichment, and increased surface runoff. Correction of these conditions might likewise be followed by at least a partial reversion to the "original" condition).

C Lakes and Reservoirs

Geological factors which significantly affect the nature of either a stream or lake include the following:

1 The geographical location of the drainage basin or watershed.

2 The size and shape of the drainage basin.

3 The general topography, i.e., mountainous or plains.

4 The character of the bedrocks and soils.

5 The character, amount, annual distribution, and rate of precipitation.

6 The natural vegetative cover of the land is, of course, responsive to and responsible for many of the above factors and is also severely subject to the whims of civilization. This is one of the major factors determining run-off versus soil absorption, etc.

D Lakes have a developmental history which somewhat parallels that of streams. This process is often referred to as natural eutrophication.

1 The methods of formation vary greatly, but all influence the character and subsequent history of the lake.

In glaciated areas, for example, a huge block of ice may have been covered with till. The glacier retreated, the ice melted, and the resulting hole became a lake. Or, the glacier may actually scoop out a hole. Landslides may dam valleys, extinct volcanoes may collapse, etc., etc.

2 Maturing or natural eutrophication of lakes.

a If not already present shoal areas are developed through erosion and deposition of the shore material by wave action and undertow.

b Currents produce bars across bays and thus cut off irregular areas.

c Silt brought in by tributary streams settles out in the quiet lake water

d Algae grow attached to surfaces, and floating free as plankton. Dead organic matter begins to accumulate on the bottom.

e Rooted aquatic plants grow on shoals and bars, and in doing so cut off bays and contribute to the filling of the lake.

f Dissolved carbonates and other materials are precipitated in the deeper portions of the lake in part through the action of plants.

g When filling is well advanced, mats of sphagnum moss may extend outward from the shore. These mats are followed by sedges and grasses which finally convert the lake into a marsh.

3 Extinction of lakes. After lakes reach maturity, their progress toward filling up is accelerated. They become extinct through:

a The downcutting of the outlet.

b Filling with detritus eroded from the shores or brought in by tributary streams.

c Filling by the accumulation of the remains of vegetable materials growing in the lake itself. (Often two or three processes may act concurrently)

III PRODUCTIVITY IN FRESH WATERS

A Fresh waters in general and under natural conditions by definition have a lesser supply of dissolved substances than marine waters, and thus a lesser basic potential for the growth of aquatic organisms. By the same token, they may be said to be more sensitive to the addition of extraneous materials (pollutants, nutrients, etc.) The following notes are directed toward natural geological and other environmental factors as they affect the productivity of fresh waters.

B Factors Affecting Stream Productivity (See Table 1)

TABLE 1

EFFECT OF SUBSTRATE ON STREAM PRODUCTIVITY*

(The productivity of sand bottoms is taken as 1)

Bottom Material	Relative Productivity
Sand	1
Marl	6
Fine Gravel	9
Gravel and silt	14
Coarse gravel	32
Moss on fine gravel	89
Fissidens (moss) on coarse gravel	111
Ranunculus (water buttercup)	194
Watercress	301
Elodea (waterweed)	452

*Selected from Tarzwell 1937

To be productive of aquatic life, a stream must provide adequate nutrients, light, a suitable temperature, and time for growth to take place.

1 Youthful streams, especially on rock or sand substrates are low in essential nutrients. Temperatures in mountainous regions are usually low, and due to the steep gradient, time for growth is short. Although ample light is available, growth of true plankton is thus greatly limited.

2 As the stream flows toward a more "mature" condition, nutrients tend to accumulate, and gradient diminishes and so time of flow increases, temperature tends to increase, and plankton flourish.

Should a heavy load of inert silt develop on the other hand, the turbidity would reduce the light penetration and consequently the general plankton production would diminish.

3 As the stream approaches base level (old age) and the time available for plankton growth increases, the balance between turbidity, nutrient levels, and temperature and other seasonal conditions, determines the overall productivity.

C Factors Affecting the Productivity of lakes (See Table 2)

1 The size, shape, and depth of the lake basin. Shallow water is more productive than deeper water since more light will reach the bottom to stimulate rooted plant growth. As a corollary, lakes with more shoreline, having more shallow water, are in general more productive. Broad shallow lakes and reservoirs have the greatest production potential (and hence should be avoided for water supplies).

TABLE 2

EFFECT OF SUBSTRATE ON LAKE PRODUCTIVITY *

(The productivity of sand bottoms is taken as 1)

Bottom Material	Relative Productivity
Sand	1
Pebbles	4
Clay	8
Flat rubble	9
Block rubble	11
Shelving rock	77

* Selected from Tarzwell 1937

2 Hard waters are generally more productive than soft waters as there are more plant nutrient minerals available. This is often greatly influenced by the character of the soil and rocks in the watershed and the quality and quantity of ground water entering the lake. In general, pH ranges of 6.8 to 8.2 appear to be most productive.

3 Turbidity reduces productivity as light penetration is reduced.

4 The presence or absence of thermal stratification with its semi-annual turnovers affects productivity by distributing nutrients throughout the water mass.

5 Climate, temperature, prevalence of ice and snow, are also of course important.

D Factors Affecting the Productivity of Reservoirs

1 The productivity of reservoirs is governed by much the same principles as that of lakes, with the difference that the water level is much more under the control of man. Fluctuations in water level can be used to deliberately increase or decrease productivity. This can be demonstrated by a comparison of the TVA reservoirs which practice a summer drawdown with some of those in the west where a winter drawdown is the rule.

2 The level at which water is removed from a reservoir is important to the productivity of the stream below. The hypolimnion may be anaerobic while the epilimnion is aerobic, for example, or the epilimnion is poor in nutrients while the hypolimnion is relatively rich.

3 Reservoir discharges also profoundly affect the DO, temperature, and turbidity in the stream below a dam. Too much fluctuation in flow may permit sections of the stream to dry, or provide inadequate dilution for toxic waste.

IV CULTURAL EUTROPHICATION

A The general processes of natural eutrophication, or natural enrichment and productivity have been briefly outlined above.

B When the activities of man speed up these enrichment processes by introducing unnatural quantities of nutrients (sewage, etc.) the result is often called cultural eutrophication. This term is often extended beyond its original usage to include the enrichment (pollution) of streams, estuaries, and even oceans, as well as lakes.

V CLASSIFICATION OF LAKES AND RESERVOIRS

A The productivity of lakes and impoundments is such a conspicuous feature that it is often used as a convenient means of classification.

1 Oligotrophic lakes are the younger, less productive lakes, which are deep, have clear water, and usually support Salmonoid fishes in their deeper waters.

2 Eutrophic lakes are more mature, more turbid, and richer. They are usually shallower. They are richer in dissolved solids; N, P, and Ca are abundant. Plankton is abundant and there is often a rich bottom fauna.

3 Dystrophic lakes, such as bog lakes, are low in Ph, water yellow to brown, dissolved solids, N, P, and Ca scanty but humic materials abundant, bottom fauna and plankton poor, and fish species are limited.

B Reservoirs may also be classified as storage, and run of the river.

1 Storage reservoirs have a large volume in relation to their inflow.

2 Run of the river reservoirs have a large flow-through in relation to their storage value.

C According to location, lakes and reservoirs may be classified as polar, temperate, or tropical. Differences in climatic and geographic conditions result in differences in their biology.

VI SUMMARY

A A body of water such as a lake, stream, or estuary represents an intricately balanced system in a state of dynamic equilibrium. Modification imposed at one point in the system automatically results in compensatory adjustments at associated points.

B The more thorough our knowledge of the entire system, the better we can judge where to impose control measures to achieve a desired result.

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Part 4. The Marine Environment and its Role in the Total Aquatic Environment

I INTRODUCTION

A The marine environment is arbitrarily defined as the water mass extending beyond the continental land masses, including the plants and animals harbored therein. This water mass is large and deep, covering about 70 percent of the earth's surface and being as deep as 7 miles. The salt content averages about 35 parts per thousand. Life extends to all depths.

B The general nature of the water cycle on earth is well known. Because the largest portion of the surface area of the earth is covered with water, roughly 70 percent of the earth's rainfall is on the seas. (Figure 1)

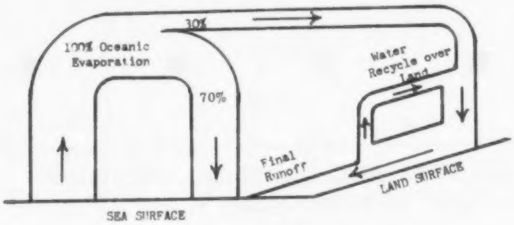

Figure 1. THE WATER CYCLE

Since roughly one third of the rain which falls on the land is again recycled through the atmosphere (see Figure 1 again), the total amount of water washing over the earth's surface is significantly greater than one third of the total world rainfall. It is thus not surprising to note that the rivers which finally empty into the sea carry a disproportionate burden of dissolved and suspended solids picked up from the land. The chemical composition of this burden depends on the composition of the rocks and soils through which the river flows, the proximity of an ocean, the direction of prevailing winds, and other factors. This is the substance of geological erosion. (Table 1)

TABLE 1

PERCENTAGE COMPOSITION OF THE MAJOR IONS OF TWO STREAMS AND SEA WATER

(Data from Clark, F.W., 1924, "The Composition of River and Lake Waters of the United States", U.S. Geol. Surv., Prof. Paper No. 135; Harvey, H.W., 1957, "The Chemistry and Fertility of Sea Waters", Cambridge University Press, Cambridge)

Ion	Delaware River at Lambertville, N.J.	Rio Grande at Laredo, Texas	Sea Water
Na	6.70	14.78	30.4
K	1.46	.85	1.1
Ca	17.49	13.73	1.16
Mg	4.81	3.03	3.7
Cl	4.23	21.65	55.2
SO_4	17.49	30.10	7.7
CO_3	32.95	11.55	+HCO_3 0.35

C For this presentation, the marine environment will be (1) described using an ecological approach, (2) characterized ecologically by comparing it with freshwater and estuarine environments, and (3) considered as a functional ecological system (ecosystem).

II FRESHWATER, ESTUARINE, AND MARINE ENVIRONMENTS

Distinct differences are found in physical, chemical, and biotic factors in going from a freshwater to an oceanic environment. In general, environmental factors are more constant in freshwater (rivers) and oceanic environments than in the highly variable and harsh environments of estuarine and coastal waters. (Figure 2)

A Physical and Chemical Factors

Rivers, estuaries, and oceans are compared in Figure 2 with reference to the relative instability (or variation) of several important parameters. In the oceans, it will be noted, very little change occurs in any parameter. In rivers, while "salinity" (usually referred to as "dissolved solids") and temperature (accepting normal seasonal variations) change little, the other four parameters vary considerably. In estuaries, they all change.

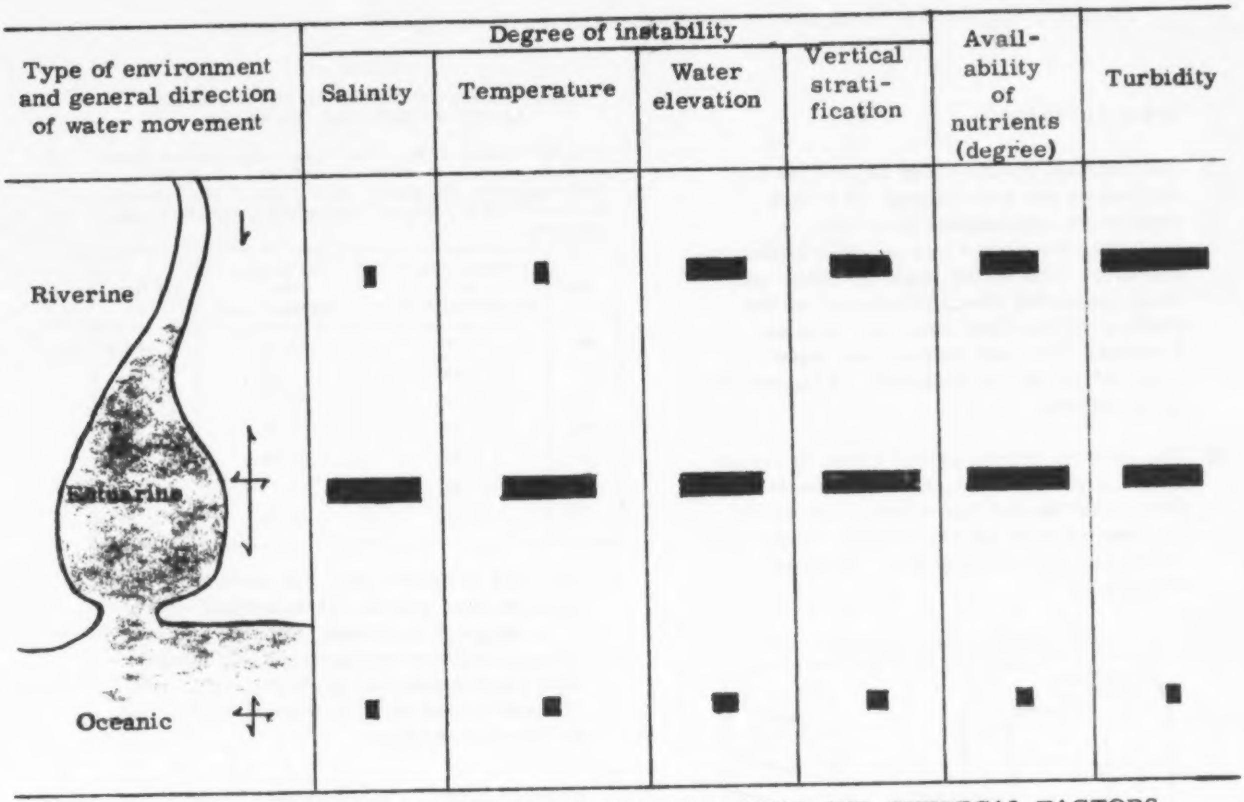

Figure 2 . RELATIVE VALUES OF VARIOUS PHYSICAL AND CHEMICAL FACTORS FOR RIVER, ESTUARINE, AND OCEANIC ENVIRONMENTS

B Biotic Factors

1 A complex of physical and chemical factors determine the biotic composition of an environment. In general, the number of species in a rigorous, highly variable environment tends to be less than the number in a more stable environment (Hedgpeth, 1966).

2 The dominant animal species (in terms of total biomass) which occur in estuaries are often transient, spending only a part of their lives in the estuaries. This results in better utilization of a rich environment.

C Zones of the Sea

The nearshore environment is often classified in relation to tide level and water depth. The nearshore and offshore oceanic regions together, are often classified with reference to light penetration and water depth. (Figure 3)

1 Neritic - Relatively shallow-water zone which extends from the high-tide mark to the edge of the continental shelf.

MARINE ECOLOGY

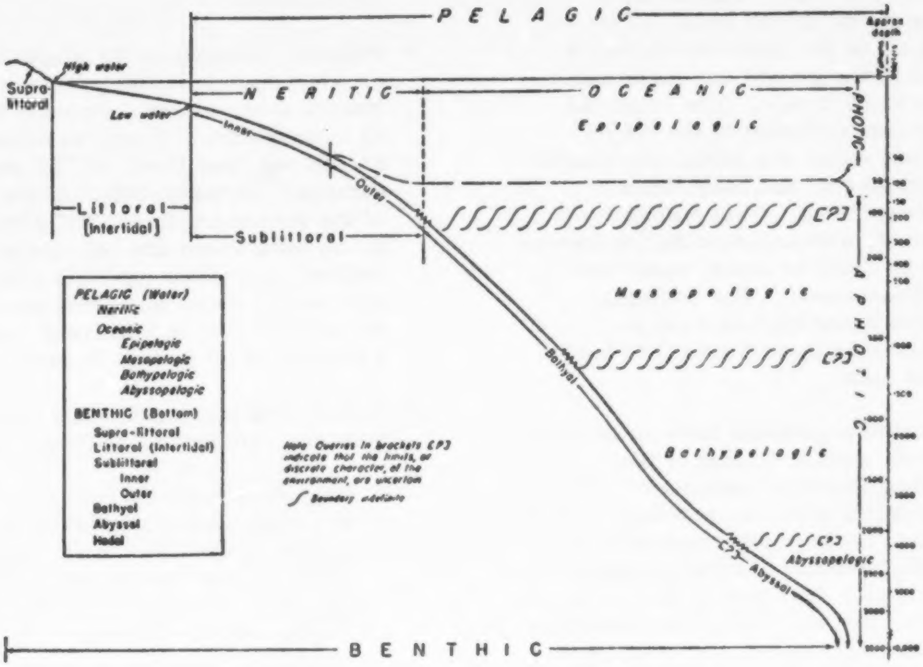
PELAGIC
Approx depth
High water
Supra-littoral
Low water
NERITIC
OCEANIC
Epipelagic
PHOTIC
Inner
Outer
Littoral (Intertidal)
Sublittoral
[?]
Mesopelagic
APHOTIC
Bathyal
[?]
Bathypelagic
[?]
Abyssopelagic
[?]
Abyssal
BENTHIC
PELAGIC (Water)
Neritic
Oceanic
Epipelagic
Mesopelagic
Bathypelagic
Abyssopelagic
BENTHIC (Bottom)
Supra-littoral
Littoral (Intertidal)
Sublittoral
Inner
Outer
Bathyal
Abyssal
Hadal
Note Queries in brackets [?] indicate that the limits, or discrete character, of the environment, are uncertain
Boundary indefinite

FIGURE 3—*Classification of marine environments*

a Stability of physical factors is intermediate between estuarine and oceanic environments.

b Phytoplankters are the dominant producers but in some locations attached algae are also important as producers.

c The animal consumers are zooplankton, nekton, and benthic forms.

2 Oceanic - The region of the ocean beyond the continental shelf. Divided into three parts, all relatively poorly populated compared to the neritic zone.

a Euphotic zone - Waters into which sunlight penetrates (often to the bottom in the neritic zone). The zone of primary productivity often extends to 183 meters below the surface.

1) Physical factors fluctuate less than in the neritic zone.

2) Producers are the phytoplankton and consumers are the zooplankton and nekton.

b Bathyal zone - From the bottom of the euphotic zone to about 2000 meters.

1) Physical factors relatively constant but light is absent.

2) Producers are absent and consumers are scarce.

c Abyssal zone - All the sea below the bathyal zone.

1) Physical factors more constant than in bathyal zone.

2) Producers absent and consumers even less abundant than in the bathyal zone.

III SEA WATER AND THE BODY FLUIDS

A Sea water is a remarkably suitable environment for living cells, as it contains all of the chemical elements essential to the growth and maintenance of plants and animals. The ratio and often the concentration of the major salts of sea water are strikingly similar in the cytoplasm and body fluids of marine organisms. This similarity is also evident, although modified somewhat in the body fluids of fresh water and terrestrial animals. For example, sterile sea water may be used in emergencies as a substitute for blood plasma in man.

B Since marine organisms have an internal salt content similar to that of their surrounding medium (isotonic condition) osmoregulation poses no problem. On the other hand, fresh water organisms are hypertonic (osmotic pressure of body fluids is higher than that of the surrounding water). Hence, fresh water animals must constantly expend more energy to keep water out (i.e., high osmotic pressure fluids contain more salts, the action being then to dilute this concentration with more water).

1 Generally, marine invertebrates are narrowly poikilosmotic, i.e., the salt concentration of the body fluids changes with that of the external medium. This has special significance in estuarine situations where salt concentrations of the water often vary considerably in short periods of time.

2 Marine bony fish (teleosts) have lower salt content internally than the external environment (hypotonic). In order to prevent dehydration, water is ingested and salts are excreted through special cells in the gills.

IV FACTORS AFFECTING THE DISTRIBUTION OF MARINE AND ESTUARINE ORGANISMS

A Salinity. Salinity is the single most constant and controlling factor in the marine environment, probably followed by temperature. It ranges around 35,000 mg. per liter, or "35 parts per thousand" (symbol: 35‰) in the language of the oceanographer. While variations in the open ocean are relatively small, salinity decreases rapidly as one approaches shore and proceeds through the estuary and up into fresh water with a salinity of "0 ‰ (see Figure 2)

B Salinity and temperature as limiting factors in ecological distribution.

1 Organisms differ in the salinities and temperatures in which they prefer to live, and in the variabilities of these parameters which they can tolerate. These preferences and tolerances often change with successive life history stages, and in turn often dictate where the organisms live· their "distribution."

2 These requirements or preferences often lead to extensive migrations of various species for breeding, feeding, and growing stages. One very important result of this is that an estuarine environment is an absolute necessity for over half of all coastal commercial and sport related species of fishes and invertebrates, for either all or certain portions of their life histories. (Part V, figure 8)

3 The Greek word roots "eury" (meaning wide) and "steno" (meaning narrow) are customarily combined with such words as "haline" for salt, and "thermal" for temperature, to give us "euryhaline" as an adjective to characterize an organism able to tolerate a wide range of salinity, for example; or "stenothermal" meaning one which cannot stand much change in temperature. "Meso-" is a prefix indicating an intermediate capacity.

C Marine, estuarine, and fresh water organisms. (See Figure 4)

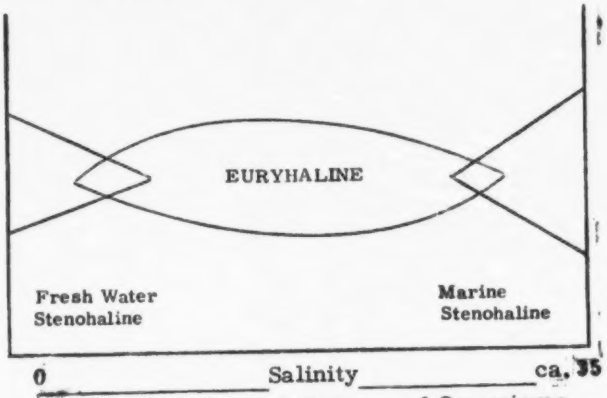

Figure 4. Salinity Tolerance of Organisms

1 Offshore marine organisms are, in general, both stenohaline and stenothermal unless, as noted above, they have certain life history requirements for estuarine conditions.

2 Fresh water organisms are also stenohaline, and (except for seasonal adaptation) meso- or stenothermal. (Figure 2)

3 Indigenous or native estuarine species that normally spend their entire lives in the estuary are relatively few in number. (See Figure 5). They are generally meso- or euryhaline and meso- or eurythermal.

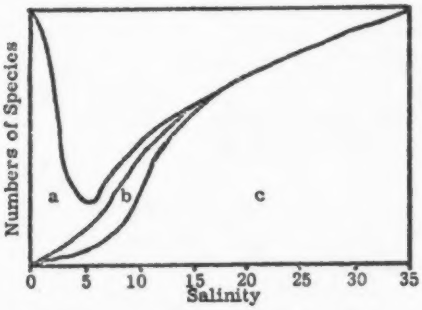

Figure 5. DISTRIBUTION OF ORGANISMS IN AN ESTUARY

a Euryhaline, freshwater
b Indigenous, estuarine, (mesohaline)
c Euryhaline, marine

4 Some will known and interesting examples of migratory species which change their environmental preferences with the life history stage include the shrimp (mentioned above), striped bass, many herrings and relatives, the salmons, and many others. None are more dramatic than the salmon hordes which hatch in headwater streams, migrate far out to feed and grow, then return to the mountain stream where they hatched to lay their own eggs before dying.

5 Among euryhaline animals landlocked (trapped), populations living in lowered salinities often have a smaller maximum size than individuals of the same species living in more saline waters. For example, the lamprey (Petromyzon marinus) attains a length of nearly one meter in the sea, while in the Great Lakes the length is one-half.

Usually the larvae of aquatic organisms are more sensitive to changes in salinity than are the adults. This characteristic both limits and dictates the distribution and size of populations.

D The effects of tides on organisms.

1 Tidal fluctuations probably subject the benthic or intertidal populations to the most extreme and rapid variations of environmental stress encountered in any aquatic habitat. Highly specialized communities have developed in this zone, some adapted to the rocky surf zones of the open coast, others to the muddy inlets of protected estuaries Tidal reaches of fresh water rivers, sandy beaches, coral reefs and mangrove swamps in the tropics; all have their own floras and faunas. All must emerge and flourish when whatever water there is rises and covers or tears at them, all must collapse or retract to endure drying, blazing tropical sun, or freezing arctic ice during the low tide interval. Such a community is depicted in Figure 6.

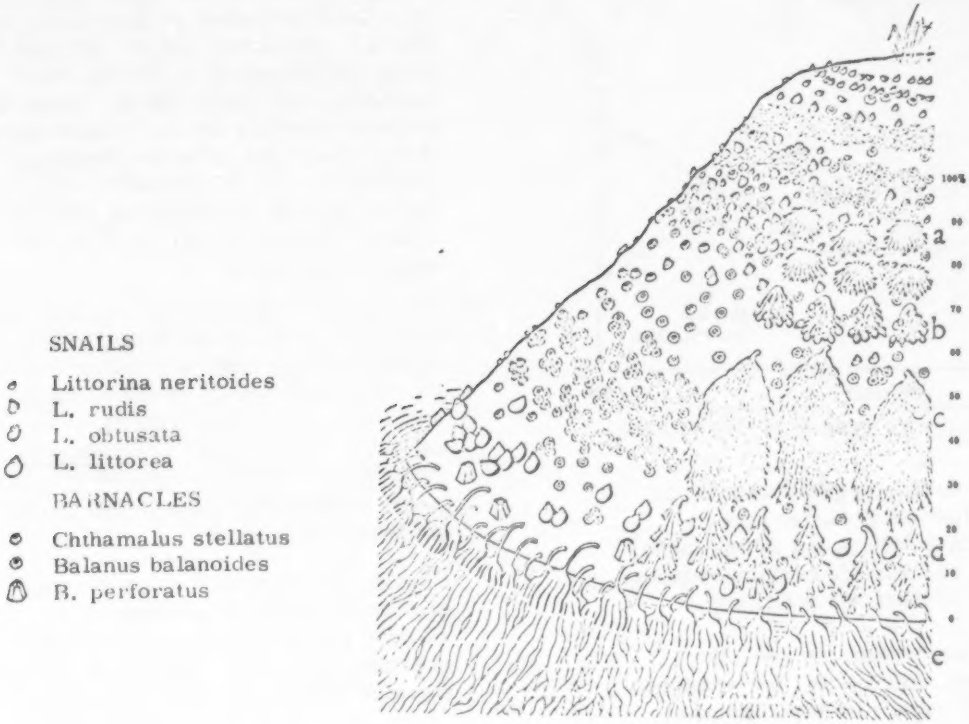
SNAILS
Littorina neritoides
L. rudis
L. obtusata
L. littorea
BARNACLES
Chthamalus stellatus
Balanus balanoides
B. perforatus
100%
90
80
70
60
50
40
30
20
10
0
a
b
c
d
e

Figure 6

Zonation of plants, snails, and barnacles on a rocky shore. While this diagram is based on the situation on the southwest coast of England, the general idea of zonation may be applied to any temperate rocky ocean shore, though the species will differ. The gray zone consists largely of lichens. At the left is the zonation of rocks with exposure too extreme to support algae; at the right, on a less exposed situation, the animals are mostly obscured by the algae. Figures at the right hand margin refer to the percent of time that the zone is exposed to the air, i.e., the time that the tide is out. Three major zones can be recognized: the Littorina zone (above the gray zone); the Balanoid zone (between the gray zone and the laminarias); and the Laminaria zone. a. Pelvetia canaliculata; b. Fucus spiralis; c. Ascophyllum nodosum; d. Fucus serratus; e. Laminaria digitata. (Based on Stephenson)

V FACTORS AFFECTING THE PRODUCTIVITY OF THE MARINE ENVIRONMENT

A The sea is in continuous circulation. Without circulation, nutrients of the ocean would eventually become a part of the bottom and biological production would cease. Generally, in all oceans there exists a warm surface layer which overlies the colder water and forms a two-layer system of persistent stability. Nutrient concentration is usually greatest in the lower zone. Wherever a mixing or disturbance of these two layers occurs biological production is greatest.

B The estuaries are also a mixing zone of enormous importance. Here the fertility washed off the land is mingled with the nutrient capacity of seawater, and many of the would's most productive waters result.

C When man adds his cultural contributions of sewage, fertilizer, silt or toxic waste, it is no wonder that the dynamic equilibrium of the ages is rudely upset, and the environmentalist cries, "See what man hath wrought"!

ACKNOWLEDGEMENT:

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Part 5: Wetlands

I INTRODUCTION

A Broadly defined, wetlands are areas which are "to wet to plough but too thick to flow." The soil tends to be saturated with water, salt or fresh, and numerous channels or ponds of shallow or open water are common. Due to ecological features too numerous and variable to list here, they comprise in general a rigorous (highly stressed) habitat, occupied by a small relatively specialized indigenous (native) flora and fauna.

B They are prodigiously productive however, and many constitute an absolutely essential habitat for some portion of the life history of animal forms generally recognized as residents of other habitats (Figure 8). This is particularly true of tidal marshes as mentioned below.

C Wetlands in toto comprise a remarkably large proportion of the earth's surface, and the total organic carbon bound in their mass constitutes an enormous sink of energy.

D Since our main concern here is with the "*aquatic*" environment, primary emphasis will be directed toward a description of wetlands as the transitional zone between the waters and the land, and how their desecration by human culture spreads degradation in both directions.

II TIDAL MARSHES AND THE ESTUARY

A "There is no other case in nature, save in the coral reefs, where the adjustment of organic relations to physical condition is seen in such a beautiful way as the balance between the growing marshes and the tidal streams by which they are at once nourished and worn away." (Shaler, 1886)

B Estuarine pollution studies are usually devoted to the dynamics of the circulating water, its chemical, physical, and biological parameters, bottom deposits, etc.

C It is easy to overlook the intimate relationships which exist between the bordering marshland, the moving waters, the tidal flats, subtidal deposition, and seston whether of local, oceanic, or riverine origin.

D The tidal marsh (some inland areas also have salt marshes) is generally considered to be the marginal areas of estuaries and coasts in the intertidal zone, which are dominated by emergent vegetation. They generally extend inland to the farthest point reached by the spring tides, where they merge into freshwater swamps and marshes (Figure 1). They may range in width from nonexistent on rocky coasts to many kilometers.

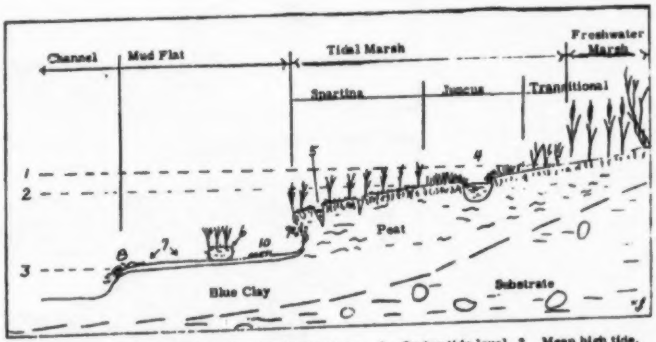

Figure 1. Zonation in a positive New England estuary. 1. Spring tide level, 2. Mean high tide, 3. Mean low tide, 4. Bog hole, 5. Ice cleavage pool, 6. Chunk of *Spartina* turf deposited by ice, 7. Organic ooze with associated community, 8. eelgrass (*Zostera*), 9. Ribbed mussels (modiolus)-clam (*mya*) - mud snail (*Nassa*) community. 10. Sea lettuce (*Ulva*)

III MARSH ORIGINS AND STRUCTURES

A In general, marsh substrates are high in organic content, relatively low in minerals and trace elements. The upper layers bound together with living roots called turf, underlaid by more compacted peat type material.

1 Rising or eroding coastlines may expose peat from ancient marsh growth to wave action which cuts into the soft peat rapidly (Figure 2).

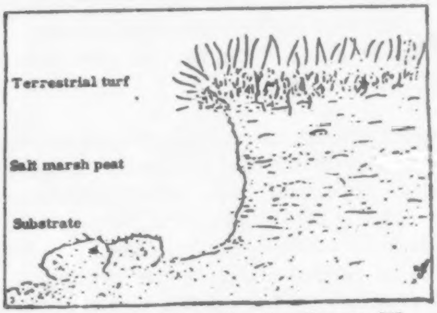

Figure 2. Diagrammatic section of eroding peat cliff

Such banks are likely to be cliff-like, and are often undercut. Chunks of peat are often found lying about on harder substrate below high tide line. If face of cliff is well above high water, overlying vegetation is likely to be typically terrestrial of the area. Marsh type vegetation is probably absent.

2 Low lying deltaic, or sinking coastlines, or those with low energy wave action are likely to have active marsh formation in progress. Sand dunes are also common in such areas (Figure 3). General coastal configuration is a factor.

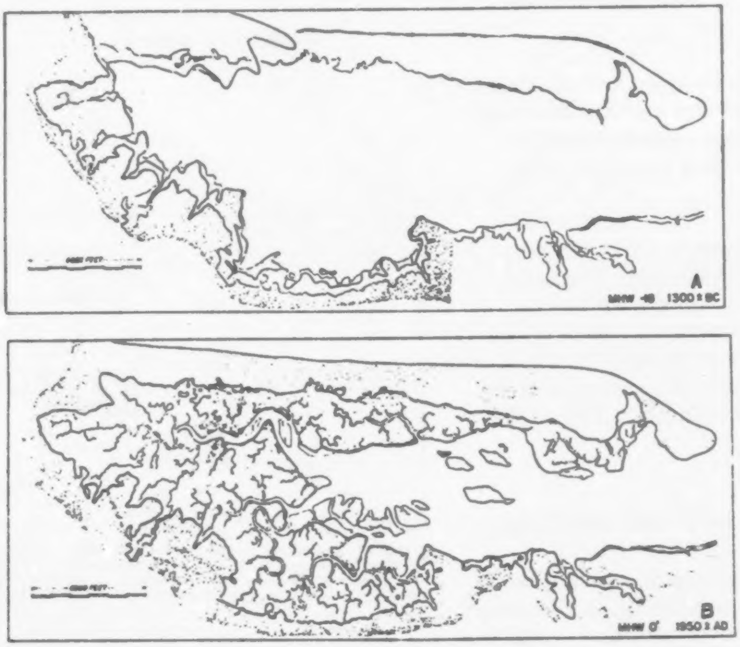

Figure 3

Development of a Massachusetts Marsh since 1300 BC, involving an 18 foot rise in water level. Shaded area indicates sand dunes. Note meandering marsh tidal drainage. A: 1300 BC, B: 1950 AD.

a Rugged or precipitous coasts or slowly rising coasts, typically exhibit narrow shelves, sea cliffs, fjords, massive beaches, and relatively less marsh area (Figure 4). An Alaskan fjord subject to recent catastrophic subsidence and rapid deposition of glacial flour shows evidence of the recent encroachment of saline waters in the presence of recently buried trees and other terrestrial vegetation, exposure of layers of salt marsh peat along the edges of channels, and a poorly compacted young marsh turf developing at the new high water level (Figure 5).

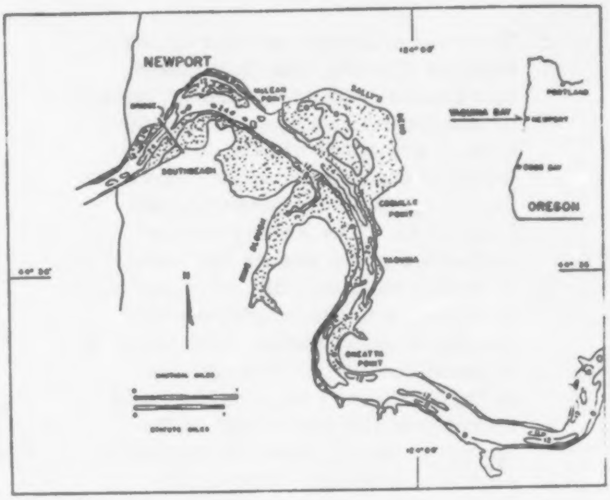

Figure 4 A River Mouth on a Slowly Rising Coast. Note absence of deltaic development and relatively little marshland, although mud flats stippled are extensive

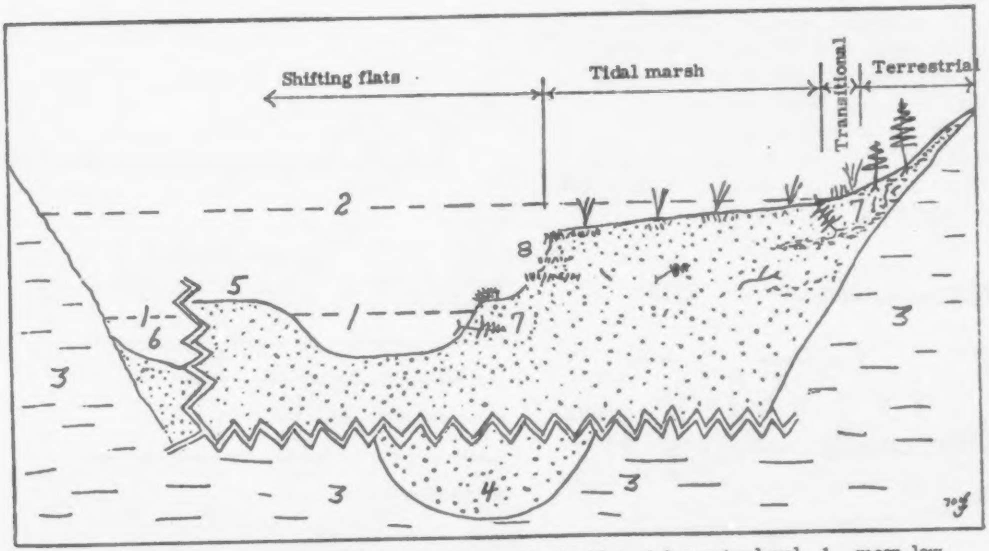

Figure 5 Some general relationships in a northern fjord with a rising water level. 1. mean low water, 2. maximum high tide, 3. Bedrock, 4. Glacial flour to depths in excess of 400 meters, 5. Shifting flats and channels, 6. Channel against bedrock, 7. Buried terrestrial vegetation, 8. Outcroppings of salt marsh peat.

b Low lying coastal plains tend to be fringed by barrier islands, broad estuaries and deltas, and broad associated marshlands (Figure 3). Deep tidal channels fan out through innumerable branching and often interconnecting rivulets. The intervening grassy plains are essentially at mean high tide level.

c Tropical and subtropical regions such as Florida, the Gulf Coast, and Central America, are frequented by mangrove swamps. This unique type of growth is able to establish itself in shallow water and move out into progressively deeper areas (Figure 6). The strong deeply embedded roots enable the mangrove to resist considerable wave action at times, and the tangle of roots quickly accumulates a deep layer of organic sediment. Mangroves in the south may be considered to be roughly the equivalent of the Spartina marsh grass in the north as a land builder. When fully developed, a mangrove swamp is an impenetrable thicket of roots over the tidal flat affording shelter to an assortment of semi-aquatic organisms such as various molluscs and crustaceans, and providing access from the nearby land to predaceous birds, reptiles, and mammals. Mangroves are not restricted to estuaries, but may develop out into shallow oceanic lagoons, or upstream into relatively fresh waters.

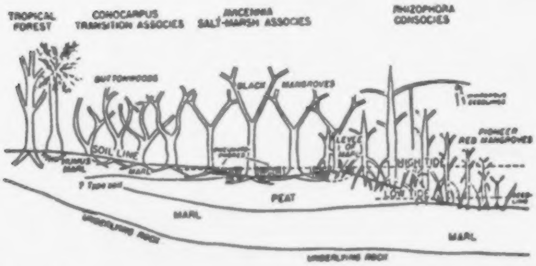

Figure 6 Diagrammatic transect of a mangrove swamp showing transition from marine to terrestrial habitat.

IV PRODUCTIVITY OF WETLANDS

A Measuring the productivity of grasslands is not easy, because today grass is seldom used directly as such by man. It is thus usually expressed as production of meat, milk, or in the case of salt marshes, the total crop of animals that obtain food per unit of area. The primary producer in a tidal marsh is the marsh grass, but very little of it is used by man as grass. (Table 1)

The nutritional analysis of several marsh grasses as compared to dry land hay is shown in Table 2.

TABLE 1. General Orders of Magnitude of Gross Primary Productivity in Terms of Dry Weight of Organic Matter Fixed Annually

Ecosystem	gms/M^2/year (grams/square meters/year)
Land deserts, deep oceans	Tens
Grasslands, forests, eutrophic lakes, ordinary agriculture	Hundreds
Estuaries, deltas, coral reefs, intensive agriculture (sugar cane, rice)	Thousands

TABLE 2. Analyses of Some Tidal Marsh Grasses

T/A	Percentage Composition					
Dry Wt.	Protein	Fat	Fiber	Water	Ash	N-free Extract
Distichlis spicata (pure stand, dry)						
2.8	5.3	1.7	32.4	8.2	6.7	45.5
Short *Spartina alterniflora* and *Salicornia europaea* (in standing water)						
1.2	7.7	2.5	31.1	8.8	12.0	37.7
Spartina alterniflora (tall, pure stand in standing water)						
3.5	7.6	2.0	29.0	8.3	15.5	37.3
Spartina patens (pure stand, dry)						
3.2	6.0	2.2	30.0	8.1	9.0	44.5
Spartina alterniflora and *Spartina patens* (mixed stand, wet)						
3.4	6.8	1.9	29.8	8.1	10.4	42.8
Spartina alterniflora (short, wet)						
2.2	8.8	2.4	30.4	8.7	13.3	36.3
Comparable Analyses for Hay						
1st cut	6.0	2.0	36.2	6.7	4.2	44.9
2nd cut	11.0	3.7	28.5	10.4	5.9	30.5

Analyses performed by Roland W. Gilbert, Department of Agricultural Chemistry, U.R.I.

B The actual utilization of marsh grass is accomplished primarily by its decomposition and ingestion by micro organisms. (Figure 7) A small quantity of seeds and solids is consumed directly by birds.

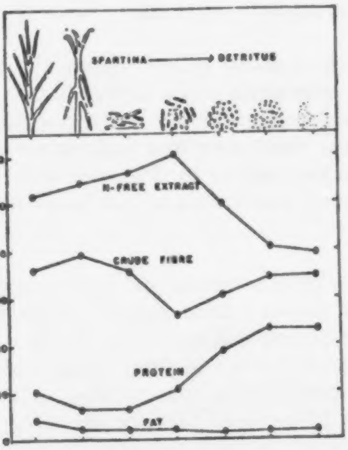

Figure 7 The nutritive composition of successive stages of decomposition of Spartina marsh grass, showing increase in protein and decrease in carbohydrate with increasing age and decreasing size of detritus particles.

1 The quantity of micro invertebrates which thrive on this wealth of decaying marsh has not been estimated, nor has the actual production of small indigenous fishes and invertebrates such as the top minnows (Fundulus), or the mud snails (Nassa), and others.

2 Many forms of oceanic life migrate into the estuaries, especially the marsh areas, for important portions of their life histories as is mentioned elsewhere (Figure 8). It has been estimated that in excess of 60% of the marine commercial and sport fisheries are estuarine or marsh dependent in some way.

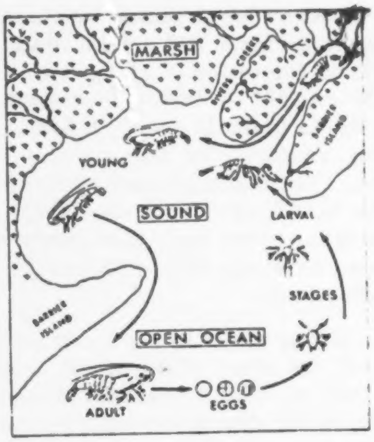

Figure 8 Diagram of the life cycle of white shrimp (after Anderson and Lunz 1965).

3 An effort to make an indirect estimate of productivity in a Rhode Island marsh was made on a single August day by recording the numbers and kinds of birds that fed on a relatively small area (Figure 9). Between 700 and 1000 wild birds of 12 species, ranging from 100 least sandpipers to uncountable numbers of seagulls were counted.

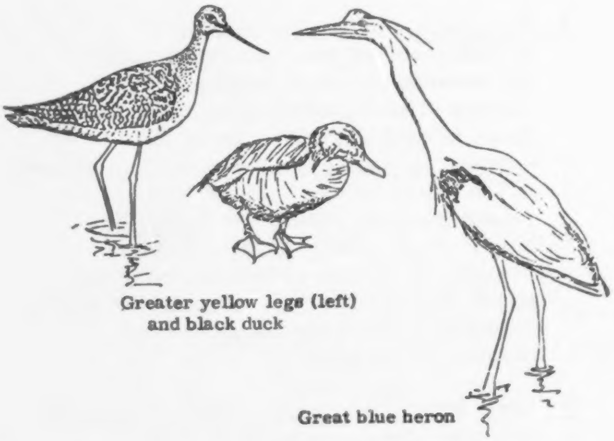

Figure 9 Some Common Marsh Birds

One-hundred black bellied plovers at approximately 283 g each would weigh on the order of 28 kg. At the same rate of food consumption, this would indicate nearly 1.8 kg. of food required for this species alone. The much greater activity of the wild birds would obviously greatly increase their food requirements, as would their relatively smaller size.

Considering the range of foods consumed, the sizes of the birds, and the fact that at certain seasons, thousands of migrating ducks and others pause to feed here, the enormous productivity of such a marsh can be better understood.

V INLAND BOGS AND MARSHES

A Much of what has been said of tidal marshes also applies to inland wetlands. As was mentioned earlier, not all inland swamps are salt-free, any more than all marshes affected by tidal rythms are saline.

B The specificity of specialized floras to particular types of wetlands is perhaps more spectacular in freshwater wetlands than in the marine, where Juncus, Spartina, and Mangroves tend to dominate.

1 Sphagnum, or peat moss, is probably one of the most widespead and abundant wetland plants on earth. Deevey (1958) quotes an estimate that there is probably upwards of 202 billions (dry weight) of metric tons of peat in the world today, derived during recent geologic time from Sphagnum bogs. Particularly in the northern regions, peat moss tends to overgrow ponds and shallow depressions, eventually forming the vast tundra plains and moores of the north.

2 Long lists of other bog and marsh plants might be cited, each with its own special requirements, topographical, and geographic distribution, etc. Included would be the familiar cattails, spike rushes, cotton grasses, sedges, trefoils, alders, and many, many others.

C Types of inland wetlands.

1 As noted above (Cf: Figure 1) tidal marshes often merge into freshwater marshes and bayous. Deltaic tidal swamps and marshes are often saline in the seaward portion, and fresh in the landward areas.

2 River bottom wetlands differ from those formed from lakes, since wide flood plains subject to periodic inundation are the final stages of the erosion of river valleys, whereas lakes in general tend to be eliminated by the geologic processes of natural eutrophication often involving Sphagnum and peat formation. Riverbottom marshes in the southern United States, with favorable climates, have luxurient growths such as the canebrake of the lower Mississippi, or a characteristic timber growth such as cypress.

3 Although bird life is the most conspicuous animal element in the fauna (Cf: Figure 9), many mammals, such as muskrats, beavers, otters, and others are also marsh-oriented. (Figure 12)

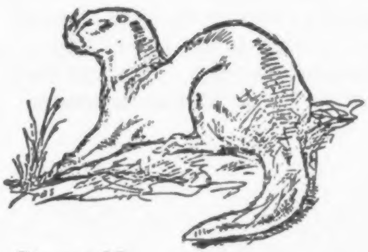

Figure 12 Otter

VI POLLUTION

A No single statement can summarize the effects of pollution on marshlands as distinct from effects noted elsewhere on other habitats.

B Reduction of Primary Productivity

The primary producers in most wetlands are the grasses and peat mosses. Production may be reduced or eliminated by:

1 Changes in the water level brought about by flooding or drainage.

a Marshland areas are sometimes diked and flooded to produce freshwater ponds. This may be for aesthetic reasons, to suppress the growth of noxious marsh inhabitating insects such as mosquitoes or biting midges, to construct an industrial waste holding pond, a thermal or a sewage stabilization pond, a "convenient" result of highway causeway construction, or other reason. The result is the elimination of an area of marsh. A small compensating border of marsh may or may not develop.

b High tidal marshes were often ditched and drained in former days to stabilize the sod for salt hay or "thatch" harvesting which was highly sought after in colonial days. This inevitably changed the character of the marsh, but it remained as essentially marshland. Conversion to outright agricultural land has been less widespread because of the necessity of diking to exclude the periodic floods or tidal incursions, and carefully timed drainage to eliminate excess precipitation. Mechanical pumping of tidal marshes has not been economical in this country, although the success of the Dutch and others in this regard is well known.

2 Marsh grasses may also be eliminated by smothering as, for example, by deposition of dredge spoils, or the spill or discharge of sewage sludge.

3 Considerable marsh area has been eliminated by industrial construction activity such as wharf and dock construction, oil well construction and operation, and the discharge of toxic brines and other chemicals.

C Consumer production (animal life) has been drastically reduced by the deliberate distribution of pesticides. In some cases, this has been aimed at nearby agricultural lands for economic crop pest control, in other cases the marshes have been sprayed or dusted directly to control noxious insects.

1 The results have been universally disastrous for the marshes, and the benefits to the human community often questionable.

2 Pesticides designed to kill nuisance insects, are also toxic to other arthropods so that in addition to the target species, such forage staples as the various scuds (amphipods), fiddler crabs, and other macroinvertebrates have either been drastically reduced or entirely eliminated in many places. For example, one familiar with fiddler crabs can traverse miles of marsh margins, still riddled with their burrows, without seeing a single live crab.

3 DDT and related compounds have been "eaten up the food chain" (biological magnification effect) until fish eating and other predatory birds such as herons and egrets (Figure 9), have been virtually eliminated from vast areas, and the accumulation of DDT in man himself is only too well known.

D Most serious of the marsh enemies is man himself. In his quest for "lebensraum" near the water, he has all but killed the water he strives to approach. Thus up to twenty percent of the marsh--estuarine area in various parts of the country has already been utterly destroyed by cut and fill real estate developments (Figures 10, 11).

E Swimming birds such as ducks, loons, cormorants, pelicans, and many others are severely jeopardized by floating pollutants such as oil.

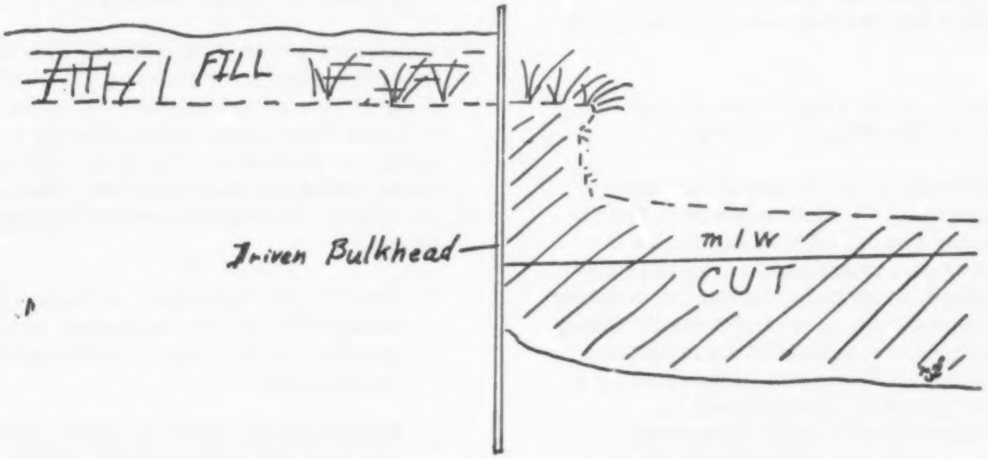
FILL
Driven Bulkhead
mlw
CUT

Figure 10. Diagrammatic representation of cut-and-fill for real estate development. mlw = mean low water

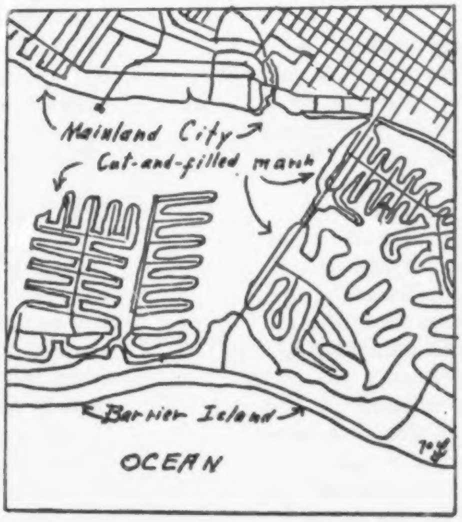
Mainland City
Cut-and-filled marsh
Barrier Island
OCEAN

Figure 11. Tracing of portion of map of a southern city showing extent of cut-and-fill real estate development.

VII SUMMARY

A Wetlands comprise the marshes, swamps, bogs, and tundra areas of the world. They are essential to the well-being of our surface waters and ground waters. They are essential to aquatic life of all types living in the open waters. They are essential as habitat for all forms of wildlife.

B The tidal marsh is the area of emergent vegetation bordering the ocean or an estuary.

C Marshes are highly productive areas, essential to the maintenance of a well rounded community of aquatic life.

D Wetlands may be destroyed by:

1 Degradation of the life forms of which it is composed in the name of nuisance control.

2 Physical destruction by cut-and-fill to create more land area.

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This outline was prepared by H. W. Jackson, former Chief Biologist, National Training Center, and revised by R. M. Sinclair, Aquatic Biologist, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Aquatic Environment, Biological Estuarine Environment, Lentic Environment, Lotic Environment, Currents, Marshes, Limnology, Magnification, Water Properties

EFFECT OF WASTEWATER TREATMENT PLANT EFFLUENT ON SMALL STREAMS

I BENTHOS ARE ORGANISMS GROWING ON OR ASSOCIATED PRINCIPALLY WITH THE BOTTOM OF WATERWAYS

Benthos is the noun.

Benthonic, benthal and benthic are adjectives.

II THE BENTHIC COMMUNITY

A Composed of a wide variety of life forms that are related because they occupy "common ground"--substrates of oceans, lakes, streams, etc. They may be attached, burrowing, or move on the interface.

1 Bacteria

A wide variety of decomposers work on organic materials, breaking them down to elemental or simple compounds.

2 Algae

Photosynthetic plants having no true roots, stems, and leaves. The basic producers of food that nurtures the animal components of the community.

3 Flowering Aquatic Plants, (Riverweeds, Pondweeds)

The largest flora, composed of complex and differentiated tissues. May be emersed, floating, or submersed according to habit.

4 Microfauna

Includes many animals that pass through a U.S. Standard Series No. 30 sieve, but are retained on a No. 100 sieve. Examples are rotifers and microcrustaceans. Some forms have organs for attachment to substrates, while others burrow into soft materials or occupy the interstices between rocks, floral or faunal materials.

5 Meiofauna

Meiofauna occupy the interstitial zone (like between sand grains) in benthic and hyporheic habitats. They are intermediate in size between the microfauna (protozoa and rotifers) and the macrofauna (insects, etc.). They pass a No. 30 sieve (0.5 mm approximately). In freshwater they include nematodes, copepods, tardigrades, naiad worms, and some flat worms. They are usually ignored in freshwater studies, since they pass the standard sieve and/or sampling devices.

6 Macrofauna (macroinvertebrates)

Animals that are retained on a No. 30 mesh sieve (0.5 mm approximately). This group includes the insects, worms, molluscs, and occasionally fish. Fish are not normally considered as benthos, though there are bottom dwellers such as sculpins, settles darters, and madtoms.

B The benthos is a self-contained community, though there is interchange with other communities. For example: Plankton settles to it, fish prey on it and lay their eggs there, terrestrial detritus and leaves are added to it, and many aquatic insects migrate from it to the terrestrial environment for their mating cycles.

C It is an in-situ water quality monitor. The low mobility of the biotic components requires that they "live with" the quality changes of the over-passing waters. Changes imposed in the long-lived components remain visible for extended periods, even after the cause has been eliminated. Only time will allow a cure for the community by drift, reproduction, and recruitment from the hyporheic zone.

D Between the benthic zone (substrate/water interface) and the underground water table is the hyporheic zone. There is considerable interchange from one zone to another.

III HISTORY OF BENTHIC OBSERVATIONS

A Ancient literature records the vermin associated with fouled waters.

B 500-year-old fishing literature refers to animal forms that are fish food and used as bait.

C The scientific literature associating biota to water pollution problems is over 100 years old (Mackenthun and Ingram, 1964).

D Early this century, applied biological investigations were initiated.

  1 The entrance of state boards of health into water pollution control activities.

  2 Creation of state conservation agencies.

  3 Industrialization and urbanization.

  4 Growth of limnological programs at universities.

E A decided increase in benthic studies occurred in the 1950's and much of today's activities are strongly influenced by developmental work conducted during this period. Some of the reasons for this are:

  1 Movement of the universities from "academic biology" to applied pollution programs.

  2 Entrance of the federal government into enforcement aspects of water pollution control.

  3 A rising economy and the development of federal grant systems.

  4 Environmental Protection Programs are a current stimulus.

IV WHY THE BENTHOS?

A It is a natural monitor

B The community contains all of the components of an ecosystem.

  1 Reducers
    a bacteria
    b fungi

  2 Producers (plants)

  3 Consumers

    a Detritivores and bacterial feeders

    b Herbivores

    c Predators

C Economy of Survey

  1 Manpower

  2 Time

  3 Equipment

D Extensive Supporting Literature

E Advantages of the Macrobenthos

  1 Relatively sessile

  2 Life history length

  3 Fish food organisms

  4 Reliability of Sampling

  5 Dollars/information

  6 Predictability

  7 Universality

  8 High hypteresis value

F "For subtle chemical changes, unequivocal data, and observations suited to some statistical evaluation will be needed. This requirement favors the macrofauna as a parameter. Macroinvertebrates are easier to sample reproductively than other organisms, numerical estimates are possible and taxonomy needed for synoptic investigations is within the reach of a non-specialist." (Wuhrmann)

G "It is self-evident that for a multitude of non-identifiable though biologically active changes of chemical conditions in rivers, small organisms with high physiological differentiation are most responsive. Thus the small macroinvertebrates (e.g. insects) are doubtlessly the most sensitive organisms for demonstrating unspecified changes of water chemistry called 'pollution'. Progress in knowledge on useful autecological properties of organisms or of transfer of such knowledge into bioassay practice has been very small in the past. Thus, the bioassay concept (relation of organisms in a stream to water quality) in water chemistry has brought not much more than visual demonstration of a few overall chemical effects. Our capability to derive chemical conditions from biological observations is, therefore, almost on the same level as fifty years ago. In the author's opinion it is idle to expect much more in the future because of the limitations inherent to natural bioassay systems (relation of organisms in a stream to water quality)." (Wuhrmann)

V REACTIONS OF THE BENTHIC MACROINVERTEBRATE COMMUNITY TO PERTURBATION

A Destruction of Organism Types

1 Beginning with the most sensitive forms, pollutants kill in order of sensitivity until the most tolerant form is the last survivor. This results in a reduction of variety or diversity of organisms.

2 The generalized order of macroinvertebrate disappearance on a sensitivity scale below pollution sources is shown in Figure 2.

Water Quality Deteriorating ↓		↑ Water Quality Improving
	Stoneflies	
	Mayflies	
	Caddisflies	
	Amphipods	
	Isopods	
	Midges	
	Oligochaetes	

As water quality improves, these reappear in the same order.

B The Number of Survivors Increase

1 Competition and predation are reduced between different species.

2 When the pollutant is a food (plants, fertilizers, animals, organic materials).

C The Number of Survivors Decrease

1 The material added is toxic or has no food value.

2 The material added produces toxic conditions as a byproduct of decomposition (e.g., large organic loadings produce an anaerobic environment resulting in the production of toxic sulfides, methanes, etc.)

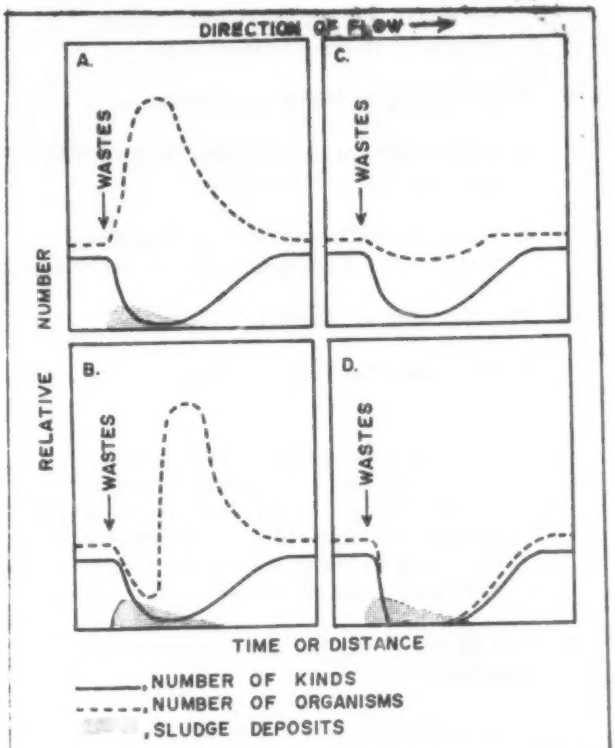
D The Effects May be Manifest in Combinations

1 Of pollutants and their effects.

2 Vary with longitudinal distribution in a stream. (Figure 1)

E Tolerance to Enrichment Grouping (Figure 2) Highly generalized

Flexibility must be maintained in the establishment of tolerance lists based on the response of organisms to the environment because of complex relationships among varying environmental conditions. Some general tolerance patterns can be established. Stonefly and mayfly nymphs, hellgrammites, and caddisfly larvae represent a grouping (sensitive or intolerant) that is generally quite sensitive to environmental changes. Blackfly larvae, scuds, sowbugs, snails, fingernail clams, dragonfly and damselfly naiads, and most kinds of midge larvae are facultative (or intermediate) in tolerance. Sludge-worms, some kinds of midge larvae (bloodworms), and some leeches are tolerant to comparatively heavy loads of organic pollutants. Sewage mosquitoes and rat-tailed maggots are tolerant of anaerobic environments for they are essentially air-breathers.



Four basic responses of bottom animals to pollution. A. Organic wastes eliminate the sensitive bottom animals and provide food in the form of sludges for the surviving tolerant forms. B. Large quantities of decomposing organic wastes eliminate sensitive bottom animals and the excessive quantities of byproducts of organic decomposition inhibit the tolerant forms; in time, with natural stream purification, water quality improves so that the tolerant forms can flourish, utilizing the sludges as food. C. Toxic materials eliminate the sensitive bottom animals; sludge is absent and food is restricted to that naturally occurring in the stream, which limits the number of tolerant surviving forms. Very toxic materials may eliminate all organisms below a waste source. D. Organic sludges with toxic materials reduce the number of kinds by eliminating sensitive forms. Tolerant survivors do not utilize the organic sludges because the toxicity restricts their growth.

Figure 1

F Structural Limitations

1 The morphological structure of a species limits the type of environment it may occupy.

a Species with complex appendages and exposed complicated respiratory structures, such as stonefly nymphs, mayfly nymphs, and caddisfly larvae, that are subjected to a constant deluge of setteable particulate matter soon abandon the polluted area because of the constant preening required to maintain mobility or respiratory functions; otherwise, they are soon smothered.

b Benthic animals in depositing zones may also be burdened by "sewage fungus" growths including stalked protozoans. Many of these stalked protozoans are host specific.

2 Species without complicated external structures, such as bloodworms and sludgeworms, are not so limited in adaptability.

a A sludgeworm, for example, can burrow in a deluge of particulate organic matter and flourish on the abundance of "manna."

b Morphology also determines the species that are found in riffles, on vegetation, on the bottom of pools, or in bottom deposits.

VI SAMPLING PROCEDURES

A Fauna

1 Qualitative sampling determines the variety of species occupying an area. Samples may be taken by any method that will capture representatives of the species present. Collections from such samplings indicate changes in the environment, but generally do not accurately reflect the degree of change. Mayflies, for example, may be reduced from 100 to 1 per square meter. Qualitative data would indicate the presence of both species, but might not necessarily delineate the change in predominance from mayflies to sludgeworms. The stop net or kick sampling technique is often used.

2 Quantitative sampling is performed to observe changes in predominance. The most common quantitative sampling tools are the Petersen, Ekman, and Ponar grabs and the Surber stream bottom sampler. Of these, the Petersen grab samples the widest variety of substrates. The Ekman grab is limited to fine-textured and soft substrates, such as silt and sludge, unless hydraulically operated.

The Surber sampler is designed for sampling riffle areas; it requires moving water to transport dislodged organisms into its net and is limited to depths of **two-thirds a meter** or less.

Kick samples of one minute duration will usually yield around 1,000 macroinvertebrates per square meter (10.5 X a one minute kick= organisms/m^2).

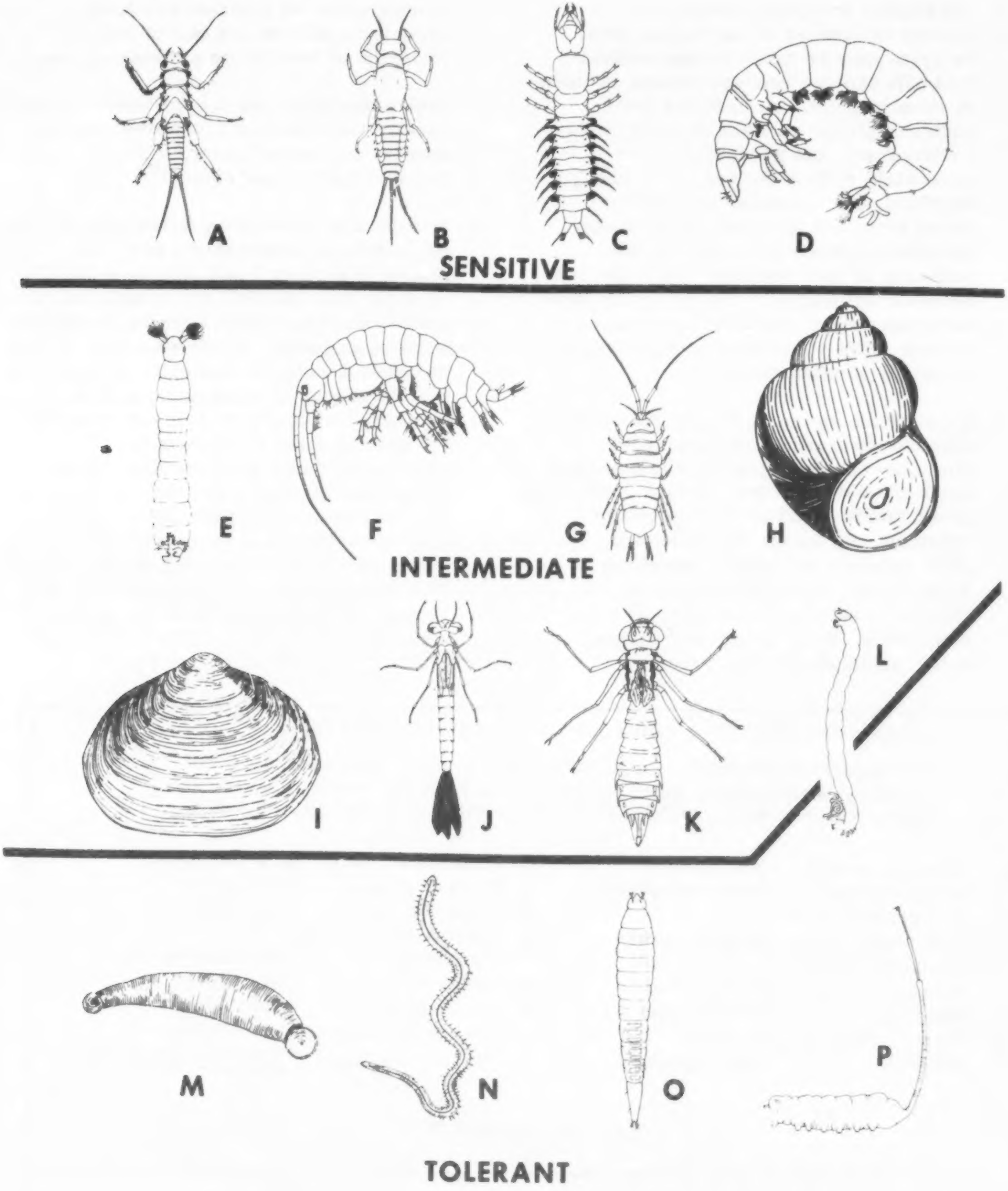
3 Manipulated substrates (often referred to as "artificial substrates") are placed in a stream and left for a specific time period. Benthic macroinvertebrates readily colonize these forming a manipulated community. Substrates may be constructed of natural materials or synthetic; may be placed in a natural situation or unnatural; and may or may not resemble the normal stream community. The point being that a great number of environmental variables are standardized and thus upstream and downstream stations may be legitimately compared in terms of water quality of the moving water column. They naturally do not evaluate what may or may not be happening to the substrate beneath said monitor. The latter could easily be the more important.

REPRESENTATIVE BOTTOM-DWELLING MACROANIMALS

Drawings from Geckler, J., K.M. Mackenthun and W.M. Ingram, 1963. Glossary of Commonly Used Biological and Related Terms in Water and Waste Water Control, DHEW, PHS, Cincinnati, Ohio, Pub. No. 999-WP-2.

A	Stonefly nymph	(Plecoptera)	I	Fingernail clam	(Sphaeriidae)
B	Mayfly nymph	(Ephemeroptera)	J	Damselfly naiad	(Zygoptera)
C	Hellgrammite or Dobsonfly larvae	(Megaloptera)	K	Dragonfly naiad	(Anisoptera)
D	Caddisfly larvae	(Trichoptera)	L	Bloodworm or midge fly larvae	(Chironomidae)
E	Black fly larvae	(Simuliidae)	M	Leech	(Hirudinea)
F	Scud	(Amphipoda)	N	Sludgeworm	(Tubificidae)
G	Aquatic sowbug	(Isopoda)	O	Sewage fly larvae	(Psychodidae)
H	Snail	(Gastropoda)	P	Rat-tailed maggot	(Tubifera-Eristalis)

KEY TO FIGURE 2


A
B
C
D
SENSITIVE
E
F
G
H
INTERMEDIATE
I
J
K
L
M
N
O
P
TOLERANT

4 Invertebrates which are part of the benthos, but under certain conditions become carried downstream in appreciable numbers, are known as Drift.

Groups which have members forming a conspicuous part of the drift include the insect orders Ephemeroptera, Trichoptera, Plecoptera and the crustacean order Amphipoda.

Drift net studies are widely used and have a proven validity in stream water quality studies.

5 The collected sample is screened with a standard sieve to concentrate the organisms; these are separated from substrate and debris, and the number of each kind of organism determined. Data are then adjusted to number per unit area, usually to number of bottom organisms per square meter.

6 Independently, neither qualitative not quantitative data suffice for thorough analyses of environmental conditions. A cursory examination to detect damage may be made with either method, but a combination of the two gives a more precise determination. If a choice must be made, quantitative sampling would be best, because it incorporates a partial qualitative sample.

7 Studies have shown that a significant number and variety of macroinvertebrates inhabit the hyporheic zone in streams. As much as 80% of the macroinvertebrates may be below 5 cm in this hyporheic zone. Most samples and sampling techniques do not penetrate the substrate below the 5 cm depth. All quantitative studies must take this and other substrate factors into account when absolute figures are presented on standing crop and numbers per square meter, etc.

B Flora

1 Direct quantitative sampling of naturally growing bottom algae is difficult. It is basically one of collecting algae from a standard or uniform area of the bottom substrates without disturbing the delicate growths and thereby distort the sample. Indirect quantitative sampling is the best available method.

2 Manipulated substrates, such as wood blocks, glass or plexiglass slides, bricks, etc., are placed in a stream. Bottom-attached algae will grow on these artificial substrates. After two or more weeks, the artificial substrates are removed for analysis. Algal growths are scraped from the substrates and the quantity measured. Since the exposed substrate area and exposure periods are equal at all of the sampling sites, differences in the quantity of algae can be related to changes in the quality of water flowing over the substrates.

VII ANALYSES OF MICROFLORA

A Enumeration

1 The quantity of algae on manipulated substrates can be measured in several ways. Microscopic counts of algal cells and dry weight of a algal material are long established methods.

2 Microscopic counts involve thorough scraping, mixing and suspension of the algal cells. From this mixture an aliquot of cells is withdrawn for enumeration under a microscope. Dry weight is determined by drying and weighing the algal sample, then igniting the sample to burn off the algal materials, leaving inert inorganic materials that are again weighed. The difference between initial dry weight and weight after ignition is attributed to algae.

3 Any organic sediments, however, that settle on the substrate along with the algae are processed also.

Thus, if organic wastes are present appreciable errors may enter into this method.

B Chlorophyll Analysis

1 During the past decade, chlorophyll analysis has become a popular method for estimating algal growth. Chlorophyll is extracted from the algae and is used as an index of the quantity of algae present. The advantages of chlorophyll analysis are rapidity, simplicity, and vivid pictorial results.

2 The algae are scrubbed from the placed substrate samples, ground, then each sample is steeped in equal volumes, 90% aqueous acetone, which extracts the chlorophyll from the algal cells. The chlorophyll extracts may be compared visually.

3 Because the cholorophyll extracts fade with time, colorimetry should be used for permanent records. For routine records, simple colorimeters will suffice. At very high cholorophyll densities, interference with colorimetry occurs, which must be corrected through serial dilution of the sample or with a nomograph.

C Autotrophic Index

The chlorophyll content of the periphyton is used to estimate the algal biomass and as an indicator of the nutrient content (or trophic status) or toxicity of the water and the taxonomic composition of the community. Periphyton growing in surface water relatively free of organic pollution consists largely of algae, which contain approximately 1 to 2 percent chlorophyll a by dry weight. If dissolved or particulate organic matter is present in high concentrations, large populations of filamentous bacteria, stalked protozoa, and other nonchlorophyll bearing microorganisms develop and the percentage of chlorophyll is then reduced. If the biomass-chlorophyll a relationship is expressed as a ratio (the autotrophic index), values greater than 100 may result from organic pollution (Weber and McFarland, 1969; Weber, 1973).

$$\text{Autotrophic Index} = \frac{\text{Ash-free Wgt (mg/m}^2\text{)}}{\text{Chlorophyll a (mg/m}^2\text{)}}$$

VIII MACROINVERTEBRATE ANALYSES

A Taxonomic

The taxonomic level to which animals are identified depends on the needs, experience, and available resources. However, the taxonomic level to which identifications are carried in each major group should be constant throughout a given study.

B Biomass

Macroinvertebrate biomass (weight of organisms per unit area) is a useful quantitative estimation of standing crop.

C Reporting Units

Data from quantitative samples may be used to obtain:

1 Total standing crop of individuals, or biomass, or both per unit area or unit volume or sample unit, and

2 Numbers of biomass, or both, of individual taxa per unit area or unit volume or sample unit.

3 Data from devices sampling a unit area of bottom will be reported in grams dry weight or ash-free dry weight per square meter (gm/m^2), or numbers of individuals per square meter, or both.

4 Data from multiplate samplers will be reported in terms of the total surface area of the plates in grams dry weight or ash-free dry weight or numbers of individuals per square meter, or both.

5 Data from rock-filled basket samplers will be reported as grams dry weight or numbers of individuals per sampler, or both.

IX FACTORS INVOLVED IN DATA INTERPRETATION

Two very important factors in data evaluation are a thorough knowledge of conditions under which the data were collected and a critical assessment of the reliability of the data's representation of the situation.

A Maximum-Minimum Values

The evaluation of physical and chemical data to determine their effects on aquatic organisms is primarily dependent on maximum and minimum obsdrved values. The mean is useful only when the data are relatively uniform. The minimum or maximum values usually create acute conditions in the environment.

B Identification

Precise identification of organisms to species requires a specialist for each taxonomic group. Many immature aquatic forms have not been associated with the adult species. Therefore, one who is certain of the genus but not the species should utilize the generic name, not a potentially incorrect species name. The method of interpreting biological data on the basis of numbers of kinds and numbers of organisms is useful.

C Lake and Stream Influence

Physical characteristics of a body of water also affect animal populations. Lakes or impounded bodies of water support different faunal associations than rivers. The number of kinds present in a lake may be less than that found in a stream because of a more uniform habitat. A lake is all pool, but a river is composed of both pools and riffles. The nonflowing water of lake exhibits a more complete settling of particulate organic matter that naturally supports a higher population of detritus consumers. For these reasons, the bottom fauna of a lake or impoundment, or stream pool cannot be directly compared with that of a flowing stream riffle.

D Extrapolation

How can bottom-dwelling macrofauna data be extrapolated to other environmental components? It must be borne in mind that a component of the total environment is being sampled. If the sampled component exhibits changes, then so must the other interdependent components of the environment. For example, a clean stream with a wide variety of desirable bottom organisms would be expected to have a wide variety of desirable bottom fishes; when pollution reduces the number of bottom organisms, a comparable reduction would be expected in the number of fishes. Moreover, it would be logical to conclude that any factor that eliminates all bottom organisms would eliminate most other aquatic forms of life. A clean stream with a wide variety of desirable bottom organisms would be expected to permit a variety of recreational, municipal and industrial uses.

E Expression of Data

1 Standing crop and taxonomic composition

Standing crop and numbers of taxa (types or kinds) in a community are highly sensitive to environmental perturbations resulting from the introduction of contaminants. These parameters, particularly standing crop, may vary considerably in unpolluted habitats, where they may range from the typically high standing crop of littoral zones of glacial lakes to the sparse fauna of torrential soft-water streams. Thus, it is important that comparisons are made only between truly comparable environments.

2 Diversity

Diversity indices are an additional tool for measuring the quality of the environment and the effect of perturbation on the structure of a community of macroinvertebrates. Their use is based on the generally observed phenomenon that relatively undisturbed environments support communities having large numbers of species with no individual species present in overwhelming abundance. If the species in such a community are ranked on the basis of their numerical abundance, there will be relatively few species with large numbers of individuals and large numbers of species represented by only a few individuals. Perturbation tends to reduce diversity by making the environment unsuitable for some species or by giving other species a competitive advantage.

3 Indicator-organism scheme ("rat-tailed maggot studies")

a For this technique, the individual taxa are classified on the basis of their tolerance or intolerance to various levels of putrescible wastes. Taxa are classified according to their presence or absence of different environments as determined by field studies. Some reduce data based on the presence or absence of indicator organisms to a simple numerical form for ease in presentation.

b "Biologists are engaging in fruitless exercise if they intend to make any decisions about indicator organisms by operating at the generic level of macroinvertebrate identifications." (Resh and Unzicker)

4 Reference station methods

Comparative or control station methods compare the qualitative characteristics of the fauna in clean water habitats with those of fauna in habitats subject to stress. Stations are compared on the basis of richness of species.

If adequate background data are available to an experienced investigator, these techniques can prove quite useful—particularly for the purpose of demonstrating the effects of gross to moderate organic contamination on the macroinvertebrate community. To detect more subtle changes in the macroinvertebrate community, collect quantitative data on numbers or biomass of organisms. Data on the presence of tolerant and intolerant taxa and richness of species may be effectively summarized for evaluation and presentation by means of line graphs, bar graphs, pie diagrams, histograms, or pictoral diagrams.

X IMPORTANT ASSOCIATED ANALYSES

A The Chemical Environment

1 Dissolved oxygen

2 Nutrients

3 Toxic materials

4 Acidity and alkalinity

5 Etc.

B The Physical Environment

1 Suspended solids

2 Temperature

3 Light penetration

4 Sediment composition

5 Etc.

XI AREAS IN WHICH BENTHIC STUDIES CAN BEST BE APPLIED

A Damage Assessment or Stream Health

If a stream is suffering from abuse the biota will so indicate. A biologist can determine damages by looking at the "critter" assemblage in a matter of minutes. Usually, if damages are not found, it will not be necessary to alert the remainder of the agency's staff.

pack all the equipment, pay travel and per diem, and then wait five days before enough data can be assembled to begin evaluation.

B By determining what damages have been done, the potential cause "list" can be reduced to a few items for emphasis and the entire "wonderful worlds" of science and engineering need not be practiced with the result that much data are discarded later because they were not applicable to the problem being investigated.

C Good benthic data associated with chemical, physical, and engineering data can be used to predict the direction of future changes and to estimate the amount of pollutants that need to be removed from the waterways to make them productive and useful once more.

D The benthic macroinvertebrates are an easily used index to stream health that citizens may use in stream improvement programs. "Adopt-a-stream" efforts have successfully used simple macroinvertebrate indices.

E The potential for restoring biological integrity in our flowing streams using macroinvertebrates has barely been touched.

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This outline was prepared by Lowell Keup, Chief, Technical Studies Branch, Division of Technical Support, EPA, Washington, D.C. 20460, and revised by R. M. Sinclair, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Aquatic Life, Benthos, Water Quality, Degradation, Environmental Effects, Trophic Level, Biological Communities, Ecological Distributions

METHODOLOGY FOR CHEMICAL ANALYSIS OF WATER AND WASTEWATER

I INTRODUCTION

This outline deals with chemical methods which are commonly performed in water quality laboratories. Although a large number of constituents or properties may be of interest to the analyst, many of the methods employed to measure them are based on the same analytical principles. The purpose of this outline is to acquaint you with the principles involved in commonly-used chemical methods to determine water quality.

II PRE-TREATMENTS

For some parameters, a preliminary treatment is required before the analysis begins. These treatments serve various purposes.

A Distillation - To isolate the constituent by heating a portion of the sample mixture to separate the more volatile part(s), and then cooling and condensing the resulting vapor(s) to recover the volatilized portion.

B Extraction - To isolate/concentrate the constituent by shaking a portion of the sample mixture with an immiscible solvent in which the constituent is much more soluble.

C Filtration - To separate undissolved matter from a sample mixture by passing a portion of it through a filter of specified size. Particles that are dissolved in the original mixture are so small that they stay in the sample solution and pass through the filter.

D Digestion - To change constituents to a form amenable to the specified test by heating a portion of the sample mixture with chemicals.

III METERS

For some parameters, meters have been designed to measure that specific constituent or property.

A pH Meters

pH (hydrogen ion concentration) is measured as a difference in potential across a glass membrane which is in contact with the sample and with a reference solution. The sensor apparatus might be combined into one probe or else it is divided into an indicating electrode (for the sample) and a reference electrode (for the reference solution). Before using, the meter must be calibrated with a solution of known pH (a buffer) and then checked for proper operation with a buffer of a different pH value.

B Dissolved Oxygen Meters

Dissolved oxygen meters measure the production of a current which is proportional to the amount of oxygen gas reduced at a cathode in the apparatus. The oxygen gas enters the electrode through a membrane, and an electrolyte solution or gel acts as a transfer and reaction media. Prior to use the meter must be calibrated against a known oxygen gas concentration.

C Conductivity Meters

Specific conductance is measured with a meter containing a Wheatstone bridge which measures the resistance of the sample solution to the transmission of an electric current. The meter and cell are calibrated according to the conductance of a standard solution of potassium chloride measured at 25°C by a "standard" cell with electrodes one cm square spaced one cm apart. This is why results are called "specific" conductance.

D Turbidimeters

A turbidimeter compares the intensity of light scattered by particles in the sample under defined conditions with the intensity of light scattered by a standard reference suspension.

IV SPECIFIC ION ELECTRODES

Just as the conventional glass electrode for pH develops an electrical potential in response to the activity of hydrogen ion in solution, the specific ion electrode develops an electrical potential in response to the activity of the ion for which the electrode is specific. The potential and activity are related according to the Nernst equation. Simple analytical techniques can be applied to convert activity to an expression of concentration.

These electrodes are used with a pH meter with an expanded mV scale or with a specific ion meter. Two examples are the ammonia and fluoride electrodes.

A Ammonia

The ammonia electrode uses a hydrophobic gas-permeable membrane to separate the sample solution from an ammonium chloride internal solution. Ammonia in the sample diffuses through the membrane and alters the pH of the internal solution, which is sensed by a pH electrode. The constant level of chloride in the internal solution is sensed by a chloride selective ion electrode which acts as the reference electrode.

B Fluoride

The fluoride electrode consists of a lanthanum fluoride crystal across which a potential is developed by fluoride ions. The cell may be represented by Ag/Ag Cl, $Cl^-(0.3)$, $F^-(0.001)$ LaF/test solution/SCE/. It is used in conjunction with a standard single junction reference electrode.

V GENERAL ANALYTICAL METHODS

A Volumetric Analysis

Titrations involve using a buret to measure the volume of a standard solution of a substance required to completely react with the constituent of interest in a measured volume of sample. One can then calculate the original concentration of the constituent of interest.

There are various ways to detect the end point when the reaction is complete.

1 Color change indicators

The method may utilize an indicator which changes color when the reaction is complete. For example, in the Chemical Oxygen Demand Test the indicator, ferroin, gives a blue-green color to the mixture until the oxidation-reduction reaction is complete. Then the mixture is reddish-brown.

Several of these color-change titrations make use of the iodometric process whereby the constituent of interest quantitatively releases free iodine. Starch is added to give a blue color until enough reducing agent (sodium thiosulfate or phenylarsine oxide) is added to react with all the iodine. At this end point, the mixture becomes colorless.

2 Electrical property indicators

Another way to detect end points is a change in an electrical property of the solution when the reaction is complete. In the chlorine titration a cell containing potassium chloride will produce a small direct current as long as free chlorine is present. As a reducing agent (phenylarsine oxide) is added to reduce the chlorine, the microammeter which measures the existing direct current registers a lower reading on a scale. By observing the scale, the end point of total reduction of chlorine can be determined because the direct current ceases.

3 Specified end points

For acidity and alkalinity titrations, the end points are specified pH values for the final mixture. The pH values are those existing when common acidity or alkalinity components have been neutralized. Thus acidity is determined by titrating the sample with a standard alkali to pH 8.2 when carbonic acid would be neutralized to $(HCO_3)^-$. Alkalinity (except for highly acidic samples) is determined by titrating the sample with a standard acid to pH 4.5 when the carbonate present has been converted to carbonic acid. pH meters are used to detect the specified end points.

B Gravimetric Procedures

Gravimetric methods involve direct weighing of the constituent in a container. An empty container is weighed, the constituent is separated from the sample mixture and isolated in the container, then the container with the constituent is weighed. The difference in the weights of the container before and after containing the constituent represents the weight of the constituent.

The type of container depends on the method used to separate the constituent from the sample mixture. In the solids determinations, the container is an evaporating dish (total or dissolved) or a glass fiber filter disc in a crucible (suspended). For oil and grease, the container is a flask containing a residue after evaporation of a solvent.

C Combustion

Combustion means to add oxygen. In the Total Organic Carbon Analysis, combustion is used within an instrument to convert carbonaceous material to carbon dioxide. An infrared analyzer measures the carbon dioxide.

VI PHOTOMETRIC METHODS

These methods involve the measurement of light that is absorbed or transmitted quantitatively either by the constituent of interest or else by a substance containing the constituent of interest which has resulted from some treatment of the sample. The quantitative aspect of these photometric methods is based on applying the Lambert-Beer Law which established that the amount of light absorbed is quantitatively related to the concentration of the absorbing medium at a given wavelength and a given thickness of the medium through which the light passes.

Each method requires preparing a set of standard solutions containing known amounts of the constituent of interest. Photometric values are obtained for the standards. These are used to draw a calibration (standard) curve by plotting photometric values against the concentrations. Then, by locating the photometric value for the sample on this standard curve, the unknown concentration in the sample can be determined.

A Atomic Absorption

Atomic Absorption (AA) instruments utilize absorption of light of a characteristic wavelength. This form of analysis involves aspirating solutions of metal ions (cations) or molecules containing metals into a flame where they are reduced to individual atoms in a ground electrical state. In this condition, the atoms can absorb radiation of a wavelength characteristic for each element. A lamp containing the element of interest as the cathode is used as a source to emit the characteristic line spectrum for the element to be determined.

The amount of energy absorbed is directly related to the concentration of the element of interest. Thus the Lambert-Beer Law applies. Standards can be prepared and tested and the resulting absorbance values can be used to construct a calibration (standard) curve. Then the absorbance value for the sample is located on this curve to determine the corresponding concentration.

Once the instrument is adjusted to give optimum readings for the element of interest, the testing of each solution can be done in a matter of seconds. Many laboratories wire recorders into their instruments to rapidly transcribe the data, thus conserving time spent on this aspect of the analysis. Atomic absorption techniques are generally used for metals and semi-metals in solution or else solubilized through some form of sample processing. For mercury, the principle is utilized but the absorption of light occurs in a flameless situation with the mercury in the vapor state and contained in a closed glass cell.

B Flame Emission

Flame emission photometry involves measuring the amount of light given off by atoms drawn into a flame. At certain temperatures, the flame raises the electrons in atoms to a higher energy level. When the electrons fall back to a lower energy level, the atoms lose (emit) radiant energy which can be detected and measured.

Again standards must be prepared and tested to prepare a calibration (standard) curve. Then the transmission value of the sample can be located on the curve to determine its concentration.
Many atomic absorption instruments can be used for flame emission photometry. Sodium and potassium are very effectively determined by the emission technique.

However, for many elements, absorption analysis is more sensitive because there are a great number of unexcited atoms in the flame which are available to absorb the radiant energy.

C Colorimetry

Colorimetric analyses involve treating standards which contain known concentrations of the constituent of interest and also the sample with reagents to produce a colored solution. The greater the concentration of the constituent, the more intense will be the resulting color.

The Lambert-Beer Law which relates the absorption of light to the thickness and concentration of the absorbing medium applies. Accordingly, a spectrophotometer is used to measure the amount of light of appropriate wavelength which is absorbed by the same thickness of each solution. The results from the standards are used to construct a calibration (standard) curve. Then the absorbance value for the sample is located on this curve to determine the corresponding concentration.

Many of the metals and several other parameters (phosphorus, ammonia, nitrate, nitrite, etc.) are determined in this manner.

VII GAS-LIQUID CHROMATOGRAPHY

Chromatography techniques involve a separation of the components in a mixture by using a difference in the physical properties of the components. Gas-Liquid Chromatography (GLC) involves separation based on a difference in the properties of volatility and solubility. The method is used to determine algicides, chlorinated organic compounds and pesticides.

The sample is introduced into an injector block which is at a high temperature (e.g. 210°C), causing the liquid sample to volatilize. An inert carrier gas transports the sample components through a liquid held in place as a thin film on an inert solid support material in a column. Sample components pass through the column at a speed partly governed by the relative solubility of each in the stationary liquid. Thus the least soluble components are the first to reach the detector. The type of detector used depends on the class of compounds involved. All detectors function to sense and measure the quantity of each sample component as it comes off the column. The detector signals a recorder system which registers a response.

As with other instrumental methods, standards with known concentrations of the substance of interest are measured on the instrument. A calibration (standard) curve can be developed and the concentration in a sample can be determined from this graph.

Gas-liquid chromatography methods are very sensitive (nanogram, picogram quantities) so only small amounts of samples are required. On the other hand, this extreme sensitivity often necessitates extensive clean-up of samples prior to GLC analysis.

VIII AUTOMATED METHODS

The increasing number of samples and measurements to be made in water quality laboratories has stimulated efforts to automate these analyses. Using smaller amounts of sample (semi-micro techniques), combining reagents for fewer measurements per analysis, and using automatic dispensers are all means of saving analytical time.

However, the term "automated laboratory procedures" usually means automatic introduction of the sample into the instrument, automatic treatment of the sample to test for a component of interest, automatic recording of data and, increasingly, automatic calculating and print-out of data. Maximum automation systems involve continuous sampling direct from the source (e.g. an in-place probe) with telemetering of results to a central computer.

Automated methods, especially those based on colorimetric methodology, are recognized for several water quality parameters including alkalinity, ammonia, nitrate, nitrite, phosphorus, and hardness.

IX SOURCES OF PROCEDURES

Details of the procedure for an individual measurement can be found in reference books. There are three particularly-recognized books of procedures for water quality measurements.

A Standard Methods(1)

The American Public Health Association, the American Water Works Association and the Water Pollution Control Federation prepare and publish "Standard Methods for the Examination of Water and Wastewater." As indicated by the list of publishers, this book contains methods developed for use by those interested in water or wastewater treatment.

B ASTM Standards(2)

The American Society for Testing and Materials publishes an "Annual Book of ASTM Standards" containing specifications and methods for testing materials. The "book" currently consists of 47 parts. The part applicable to water was formerly Part 23. It is now Part 31, Water.

The methods are chosen by approval of the membership of ASTM and are intended to aid industry, government agencies and the general public. Methods are applicable to industrial waste waters as well as to other types of water samples.

C EPA Methods Manual(3)

The United States Environmental Protection Agency publishes a manual of "Methods for Chemical Analysis of Water and Wastes."

EPA developed this manual to provide methodology for monitoring the quality of our Nation's waters and to determine the impact of waste discharges. The test procedures were carefully selected to meet these needs, using Standard Methods and ASTM as basic references. In many cases, the EPA manual contains completely described procedures because they modified methods from the basic references. Otherwise, the manual cites page numbers in the two references where the analytical procedures can be found.

X ACCURACY AND PRECISION

A Of the Method

One of the criteria for choosing methods to be used for water quality analysis is that the method should measure the desired property or constituent with precision, accuracy, and specificity sufficient to meet data needs. Standard references, then, include a statement of the precision and accuracy for the method which is obtained when (usually) several analysts in different laboratories used the particular method.

B Of the Analyst

Each analyst should check his own precision and accuracy as a test of his skill in performing a test. According to the U. S. EPA Handbook for Analytical Quality Control(4), he can do this in the following manner.

To check precision, the analyst should analyze samples with four different concentrations of the constituent of interest, seven times each. The study should cover at least two hours of normal laboratory operations to allow changes in conditions to affect the results. Then he should calculate the standard deviation of each of the sets of seven results and compare his values for the lowest and highest concentrations tested with the standard deviation value published for that method in the reference book. **(It may be stated as % relative standard deviation. If so, calculate results in this form.) An individual should have better values than those averaged from the work of several analysts.**

To check accuracy, he can use two of the samples used to check precision by adding a known amount (spike) of the particular constituent in quantities to double the lowest concentration used, and to bring an intermediate concentration to approximately 75% of the upper limit of application of the method. He then analyzes each of the spiked samples seven times, then calculates the average of each set of seven results. To calculate accuracy in terms of % recovery, he will also need to calculate the average of the results he got when he analyzed the unspiked samples (background). Then:

$$\% \text{ Recovery} = \left[\frac{\text{observed - background}}{\text{spike}}\right] \times 100$$

The actual calculation involves volume-concentration calculations for each term.

If the published accuracy is stated as % bias, subtract 100% from % recovery to compare results. Again, the individual result should be better than the published figure derived from the results of several analysts.

C Of Daily Performance

Even after an analyst has demonstrated his personal skill in performing the analysis, a daily check on precision and accuracy should be done. About one in every ten samples should be a duplicate to check precision and about one in every ten samples should be spiked to check accuracy.

It is also beneficial to participate in inter-laboratory quality control programs. The U.S. EPA provides reference samples at no charge to laboratories. These samples serve as independent checks on reagents, instruments or techniques; for training analysts or for comparative analyses within the laboratory. There is no certification or other formal evaluative function resulting from their use.

XI SELECTION OF ANALYTICAL PROCEDURES

Standard sources$^{(1, 2, 3)}$ will, for most parameters, contain more than one analytical procedure. Selection of the procedure to be used in a specific instance involves consideration of the use to be made of the data. In some cases, one must use specified procedures. In others, one may be able to choose among several methods.

A NPDES Permits and State Certifications

A specified analytical procedure must be used when a waste constituent is measured:

1 For an application for a National Pollutant Discharge Elimination System (NPDES) permit under Section 402 of the Federal Water Pollution Control Act (FWPCA), as amended.

2 For reports required to be submitted by dischargers under NPDES.

3 For certifications issued by States pursuant to Section 401 of the FWPCA, as amended.

Analytical procedures to be used in these situations must conform to those specified in Title 40, Chapter 1, Part 136, of the Code of Federal Regulations (CFR). The listings in the CFR usually cite two different procedures for a particular measurement.

The CFR also provides a system of applying to EPA for permission to use methods not cited in the CFR. Approval of alternative methods for nationwide use will be published in the Federal Register.

B Ambient Water Quality Monitoring

For Ambient Water Quality Monitoring, analytical procedures have not been specified by regulations. However, the selection of procedures to be used should receive attention. Use of those listed in the CFR is strongly recommended. If any of the data obtained is going to be used in connection with NPDES permits, or may be used as evidence in a legal proceeding, use of procedures listed in the CFR is again strongly recommended.

C Drinking Water Monitoring

In December, 1975, National Interim Primary Drinking Water Regulations to be effective June 24, 1977 were published in the Federal Register in Title 40, Chapter 1, Subchapter D, Part 141. The publication includes specification of analytical procedures to be used when determining compliance with the maximum contaminant levels of required parameters.

Because of the low concentrations involved in the regulations, there is often just one analytical method cited for each parameter.

Individuals or organizations may apply to EPA for permission to use methods not cited in the above. Approval of alternative methods for nationwide use will be published in the Federal Register.

XII FIELD KITS

Field kits have been devised to perform analyses outside of the laboratory. The kit may contain equipment and reagents for only one test or for a variety of measurements. It may be purchased or put together by an agency to serve its particular needs.

Since such kits are devised for performing tests with minimum time and maximum simplicity, the types of labware and reagents employed usually differ significantly from the equipment and supplies used to perform the same measurement in a laboratory.

A Shortcomings

Field conditions do not accommodate the equipment and services required for pretreatments like distillation and digestion. Nor is it practical to carry and use calibrated glassware like burets and volumetric pipets. Other problems are preparation, transport and storage of high quality reagents, of extra supplies required to test for and remove sample interferences before making the measurement, and of instruments which are very sensitive in detecting particular constituents. One just cannot carry and set up laboratory facilities in the field which are equivalent to stationary analytical facilities.

B Uses

Even though the results of field tests are usually not as accurate and precise as those performed in the laboratory, such tests do have a place in water quality programs.

In situations where only an estimate of the concentrations of various constituents is required, field tests serve well. They are invaluable sources of information for planning a full-scale sampling/testing program when decisions must be made regarding location of sampling sites, schedule of sample collection, dilution of samples required for analysis, and treatment of samples required to remove interferences to analyses.

C NPDES Permits and State Certification

Kit methods are not approved for obtaining data required for NPDES permits or State construction certifications. If one judges that such a method is justifiable for these purposes, he must apply to EPA for permission to use it.

D Drinking Water Monitoring

The DPD test kit for residual chlorine is approved in the December, 1975 Federal Register for monitoring drinking water in cases where chlorine tests are substitutes for microbiological tests.

REFERENCES

1 Standard Methods for the Examination of Water and Wastewater, 14th Edition. 1976, APHA-AWWA-WPCF, 1015 18th Street, N.W., Washington, D.C. 20036

2 1975 Annual Book of ASTM Standards, Part 31, Water. ASTM, 1916 Race Street, Philadelphia, PA 19103.

3 Methods for Chemical Analysis of Water and Wastes. 1979, U. S. EPA, EMSL, Cincinnati, OH 45268.

4 Handbook for Analytical Quality Control in Water and Wastewater Laboratories, 1972. U. S. EPA, EMSL, Cincinnati, Ohio 45268.

This outline was prepared by Audrey Kroner, Chemist, National Training and Operational Technology Center, OWPO, U. S. EPA, Cincinnati, Ohio 45268.

Descriptors: Analysis, Chemical Analysis, Methodology, Wastewater, Water Analysis

SPECIFIC CONDUCTANCE

I INTRODUCTION

An electrical conductivity measurement of a solution determines the ability of the solution to conduct an electrical current. Very concentrated solutions have a large population of ions and transmit current easily or with small resistance. Since resistivity is inversely related to conductivity $K = \frac{1}{R}$, a very concentrated solution has a very high electrical conductivity.

Electrical conductivity is determined by transmitting an electrical current through a given solution, using two electrodes. The resistance measured is dependent principally upon the ionic concentration, ionic charge, and temperature of the solution although electrode characteristics (surface area and spacing of electrodes) is also critical. Early experiments in standardizing the measurement led to construction of a "standard cell" in which the electrodes were spaced exactly 1 cm and each had a surface area of 1 cm^2. Using this cell, electrical conductivity is expressed as "Specific Conductance". Modern specific conductance cells do not have the same electrode dimensions as the early standard cell but have a characteristic electrode spacing/area ratio known as the "cell constant".

$$K_{sp} = \frac{1}{R} \times \frac{\text{distance (cm)}}{\text{area (cm}^2\text{)}}, \quad K_{sp} = \frac{1}{R} \times k$$

$$k = \text{cell constant}$$

Specific conductance units are Mhos/cm or reciprocal ohms/cm. Most natural, fresh waters in the United States have specific conductances ranging from 10 to 1,000 micromhos/cm. (1 micromho = 10^{-6} mho).

II CONDUCTIVITY INSTRUMENTS

Nearly all of the commercial specific conductance instruments are of a bridge circuit design, similar to a Wheatstone Bridge. Null or balance is detected either by meter movement, electron "ray eye" tubes, or headphones. Since resistance is directly related to temperature, some instruments have automatic temperature compensators, although inexpensive models generally have manual temperature compensation.

Conductivity instruments offer direct specific conductance readout when used with a cell "matched" to that particular instrument.

Electrodes within the cell may become damaged or dirty and accuracy may be affected; therefore, it is advisable to frequently check the instrument readings with a standard KCl solution having a known specific conductance.

III CONDUCTIVITY CELLS

Several types of conductivity cells are available, each having general applications. Dip cells are generally used for field measurement, flow cells for measurement within a closed system, and pipet cells for laboratory use. Many modifications of the above types are available for specialized laboratory applications; the Jones cells and inductive capacitance cells are perhaps the most common.

Examples of various cell ranges for the RB3 - Industrial Instruments model (0-50 micromhos/cm scale range) are in Table 1.

Cell Number	Relative Conductivity Value	Maximum range micromhos/cm	Most accurate range micromhos/cm
Cel VSO2	1	0 - 50	2 - 30
Cel VS2	10	0 - 500	20 - 300
Cel VS2O	100	0 - 5000	200 - 3000

Table 1

IV Computation of Calibration Constant

A calibration constant is a factor by which scale readings must be multiplied to compute specific conductance.

$K_{sp} = cM$

where K_{sp} = actual specific conductance

c = calibration constant

M = meter reading

For example, a 0.001 N KCl solution (147 micromhos/cm standard) may show a scale reading of 147.

$$147 = c\ 147,\ c = \frac{147}{147} = 1.00$$

In this case the cell is perfectly "matched" to the instrument, the calibration constant is 1.00, and the scale reading represents actual specific conductance. A variety of cells, each covering a specific range, may be used with any one instrument. However, a calibration constant for each cell must be computed before solutions of unknown specific conductance can be determined.

V RELATIONSHIP OF SPECIFIC CONDUCTANCE TO IONIC CONCENTRATION

Natural water consists of many chemical constituents, each of which may differ widely in ionic size, mobility, and solubility. Also, total constituent concentration and proportions of certain ions in various natural waters range considerably. However, it is surprising that for most natural waters having less than 2,000 mg/L. dissolved solids, dissolved solids values are closely related to specific conductance values, ranging in a ratio of .62 to .70. Of course this does not hold true for certain waters having considerable amounts of nonionized soluble materials, such as organic compounds and nonionized, colloidal inorganics.

Properties of some inorganic ions in regard to electrical conductivity are shown below:

Ion	micromhos/cm per meq/L conc.
Calcium	52.0
Magnesium	46.6
Sodium	48.9
Potassium	72.0
Bicarbonate	43.6
Carbonate	84.6
Chloride	75.9

VI ESTIMATION OF CONSTITUENT CONCENTRATIONS

Generally speaking, for waters having a dissolved solids concentration of less than 1,000 mg/L, calcium and magnesium (total hardness), sodium, bicarbonate and carbonate (total alkalinity), and sulfate are the principal or most abundant ions, representing perhaps 90-99% of the total ionic concentration of the water. Specific conductance, total hardness and total alkalinity are all simple and expedient measurements which can be performed in the field. Therefore, the remaining principal ions are sodium and sulfate, and concentrations of these can be estimated by empirical methods. For example, we find that a certain water has:

K_{sp} = 500 micromhos/cm
Total Hardness = 160 mg/L or 3.20 meq/L
Total Alkalinity = 200 mg/L or 3.28 meq/L, as bicarbonate.

Next we multiply the specific conductance by *0.011 (500 × 0.011 = 5.50) to estimate the total ionic concentration in meq/L.

* This factor may vary slightly for different waters

Cations (meq/L)	Anions (meq/L)
Calcium 3.20	Carbonate 0.00
Magnesium	Bicarbonate 3.28
Sodium 5.50-3.20 = 2.30	Sulfate 5.50-3.28 = 2.22
Total Cations 5.50	Total Anions 5.50

Realizing that several variables are involved in empirical analysis, application rests entirely upon testing the formula with previous complete laboratory analyses for that particular water. If correlation is within acceptable limits, analytical costs may be substantially reduced. Empirical analysis can also be used in determination of proper aliquots (dilution factor) necessary for laboratory analysis.

Records of laboratory chemical analysis may indicate that a particular stream or lake shows a characteristic response to various streamflow rates or lake water levels. If the water's environment has not been altered and water composition responds solely to natural causes, a specific conductivity measurement may be occasionally used in substitution for laboratory analyses to determine water quality. Concentration of individual constituents can thus be estimated from a specific conductance value.

VII APPLICATIONS FOR SPECIFIC CONDUCTANCE MEASUREMENTS

A Laboratory Operations(2)

- 1 Checking purity of distilled and deionized water
- 2 Estimation of dilution factors for samples
- 3 Quality control check on analytical accuracy
- 4 An electrical indicator

B Agriculture

- 1 Evaluating salinity
- 2 Estimating Sodium Adsorption Ratio

C Industry(3)

- 1 Estimating corrosiveness of water in steam boilers
- 2 Efficiency check of boiler operation

D Geology

- 1 Stratigraphic identification and characterization
  - a geological mapping
  - b oil explorations

E Oceanography

- 1 Mapping ocean currents
- 2 Estuary studies

F Hydrology

- 1 Locating new water supplies
  - a buried stream channels (See Fig. 1)
  - b springs in lakes and streams (See Fig. 2)
- 2 Detection and regulation of sea water encroachment on shore wells

G Water Quality Studies

- 1 Estimation of dissolved solids(2) (See Section V, also Fig. 3)
- 2 Empirical analysis of constituent concentrations (See Section VI, also reference 2)
- 3 Quality control check for salt water conversion studies
- 4 Determination of mixing efficiency of streams (See Fig. 4)
- 5 Determination of flow pattern of polluted currents (See Fig. 4)
- 6 Identification of significant fluctuations in industrial wastewater effluents.
- 7 Signal of significant changes in the composition of influents to waste treatment plants

FIGURE 1

DETECTION OF BURIED STREAM CHANNELS

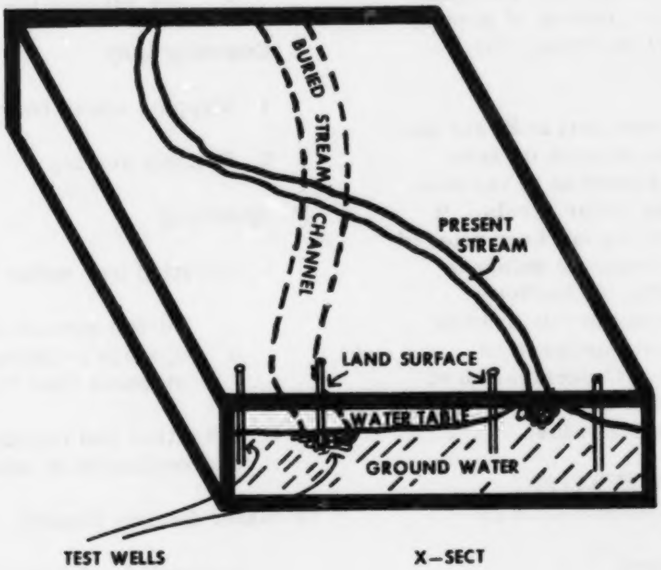
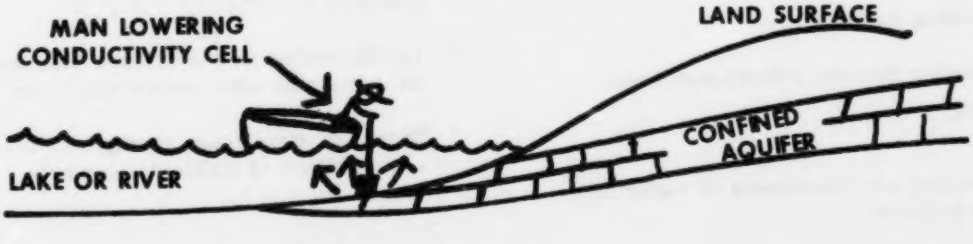

BURIED STREAM CHANNEL
PRESENT STREAM
LAND SURFACE
WATER TABLE
GROUND WATER
TEST WELLS
X-SECT

FIGURE 2

DETECTION OF SPRINGS IN LAKES AND STREAMS


MAN LOWERING CONDUCTIVITY CELL
LAND SURFACE
CONFINED AQUIFER
LAKE OR RIVER

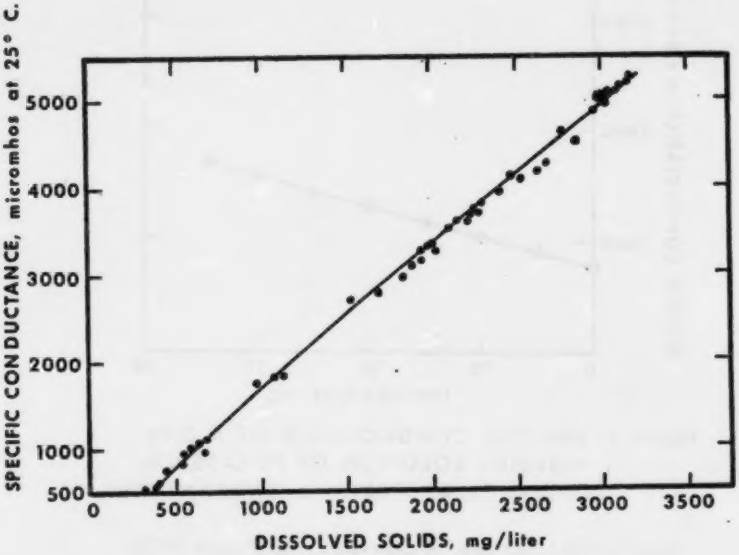


Figure 3. SPECIFIC CONDUCTANCE AND DISSOLVED SOLIDS IN COMPOSITES OF DAILY SAMPLES. , GILA RIVER AT BYLAS, ARIZONA, OCTOBER 1, 1944 TO SEPTEMBER 30, 1944.

Geological Survey Water-Supply Paper 1473.

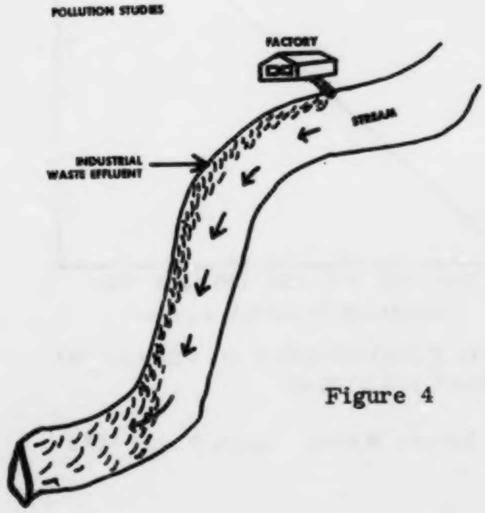

POLLUTION STUDIES
FACTORY
STREAM
INDUSTRIAL WASTE EFFLUENT

Figure 4

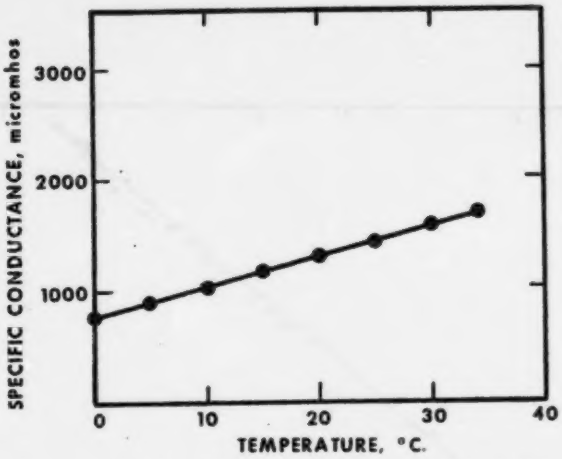


Figure 5. SPECIFIC CONDUCTANCE OF A 0.01 NORMAL SOLUTION OF POTASSIUM CHLORIDE AT VARIOUS TEMPERATURES.

Geological Survey Water-Supply Paper 1473.

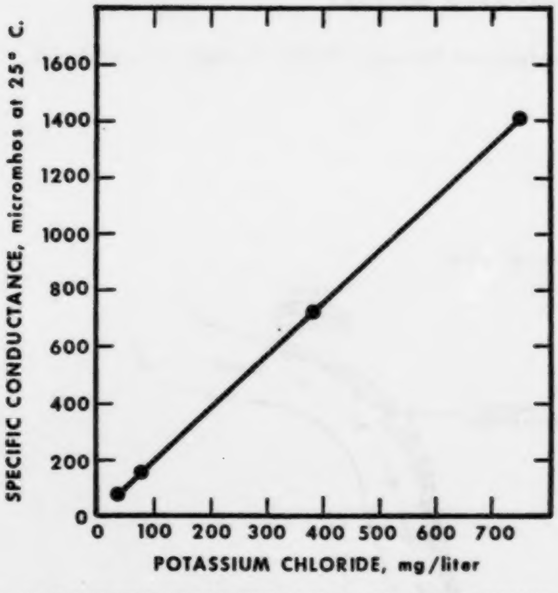


Figure 6. SPECIFIC CONDUCTANCE OF POTASSIUM CHLORIDE SOLUTIONS.

Geological Survey Water-Supply Paper 1473

VIII NPDES METHODOLOGY

A The Federal Register "List of Approved Test Procedures" for NPDES requirements specifies that specific conductance be measured with a self-contained conductivity meter, Wheatstone bridge type.(1) (2) (3)

Temperature directly affects specific conductance values (see Fig. 5). For this reason, samples should preferably be analyzed at 25°C. If not, temperature corrections should be made and results reported as μmhos/cm at 25°C.

1 The instrument should be standardized using KCl solutions. (See Fig. 6)

2 It is essential to keep the conductivity cell clean.

B The EPA manual specifies using the procedure as described in Standard Methods(2) or in ASTM Standards(3). These are approved in 40 CFR136 for NPDES Report purposes.

C Precision and Accuracy(1)

Forty-one analysts in 17 laboratories analyzed 6 synthetic water samples containing the following K_{sp} increments of inorganic salts: 100, 106. 808, 848, 1640 and 1710 micromhos/cm.

The standard deviation of the reported values was 7.55, 8.14, 66.1, 79.6, 106 and 119 μmhos/cm respectively.

The accuracy of the reported values was -2.0, -0.8, -29.3, -38.5, -87.9 and -86.9 μmhos/cm bias respectively.

REFERENCES

1 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, Cincinnati, Ohio 45268, 1974.

2 Standard Methods for the Examination of Water and Wastewater, APHA-AWWA-WPCF, 14th Edition, 1976.

3 ASTM Annual Book of Standards, Part 31, 1975.

This outline was prepared by John R. Tilstra, Chemist, National Eutrophication Research Program, Corvallis, Oregon with additions by Audrey D. Kroner, Chemist, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Chemical Analysis, Concentration, Conductivity, Dissolved Solids, Electrical Conductance, Ions, Physical Properties, Salinity, Sodium, Specific Conductivity, Sulfates, Water Analysis, Water Supplies.

CALIBRATION AND USE OF A CONDUCTIVITY METER

I EQUIPMENT AND REAGENTS

A Equipment

1 Solu Bridge conductivity meters

2 Probes

a Cell VSO2

b Cell VS2

c Cell VS2O

3 Thermometers

4 400 ml beakers

B Reagents

1 Standard KC1 solutions

Normality of KC1 Solution	Specific Conductance micromhos/cm.
0.0001	14.9
0.001	147.0
0.01	1413.0
0.1	12900.0

2 Distilled water

II CHECKING THE INSTRUMENT

A The measurement of specific conductivity as presented in sections II and III is written for one type of conductivity meter and probe.

B A battery check is made by depressing the Battery Check switch, and at the same time pressing the on-off button. The meter needle should deflect to the right (positive) and come to rest in the green zone.

C Place a 10,000 ohm resistor in the holes of the electrical contacts on the meter. Turn the temperature knob to read 25°C. Depress the on-off button and bring the meter needle to a reading of 0 by turning the specific conductance switch. The specific conductance reading should be approximately 200 micromhos/cm.

III DETERMINATION OF THE CALIBRATION CONSTANT

A Determine the temperature of the standard KC1 solutions and move the temperature knob to that value.

B Connect probe Cell VS02 to the conductivity meter.

C Rinse the probe in the beaker of distilled water, wipe the excess water with a kimwipe and place probe in the first beaker of KC1 solution (0.0001 N).

D Make certain the cell is submerged to a point at least 1/2 inch above the air hole and that no entrapped air remains. The cell should also be at least 1/2 inch from the inside walls of the flask.

E Press and hold down the ON-OFF button, simultaneously rotating the main scale knob until the meter reads zero. Release the button. (If the meter needle remains off scale or cannot be nulled, discontinue testing in that solution.)

F Record the scale reading in Table 1 and proceed to KC1 solutions 0.001N, 0.01N, 0.1 N using Steps C, D and E.

G Repeat steps C through F using the VS2, then the VS20 probe.

H Compute the cell calibration constant-a factor by which scale readings must be multiplied to compute specific conductance:

$K_{sp} = cM$

where K_{sp} = actual specific conductance,

c = calibration constant

M= meter reading

(continued next page)

Cell Number	Relative Conductivity Value	Maximum range micromhos/cm	Most accurate range micromhos/cm
Cell VSO2	1	0 - 50	2 - 30
Cell VS2	10	0 - 500	20 - 300
Cell VS2O	100	0 - 5000	200 - 3000

TABLE I DATA FOR CALIBRATION CONSTANTS

Probe	Cell VSO2				Cell VS2				Cell VS2O			
KCl Solutions	0.0001N	0.001N	0.01N	0.1N	0.0001N	0.001N	0.01N	0.1N	0.0001N	0.001N	0.01N	0.1N
Test # 1												
Cell Constant												

For each cell, calculate the cell constant by using the meter reading closest to the 400 - 600 range. The known specific conductance for the corresponding KCl solution can be found in IB Reagents. Record the cell constants on Table I.

IV DETERMINATION OF K_{sp} FOR SAMPLES

Obtain meter readings, M, for samples A, B and C using Section III C, D and E. Record M in Table 2. See Table I for the appropriate cell constant, c, to calculate K_{sp} for each sample where $K_{sp} = cM$. Record results in Table 2.

V EPA METHODOLOGY

The current EPA Manual(1) specifies using the procedures found in References 2 and 3. These procedures have been adapted for this laboratory session and all are approved in 40CFR136 for NPDES report purposes.

ACKNOWLEDGMENT

This outline contains certain portions of previous outlines by Messrs. J. W. Mandia, and J. R. Tilstra.

REFERENCES

1 Methods for Chemical Analysis of Water and Wastes, USEPA, AQCL, Cincinnati, OH 45268, 1974.

2 Standard Methods for the Examination of Water and Wastewater, 14th Edition. 1976.

3 Book of ASTM Standards, Part 31, 1975.

This outline was prepared by C. R. Feldmann, Chemist, National Training and Operational Technology Center, and revised by Audrey D. Kroner, also with National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Analytical Techniques, Conductivity, Electrical Conductance, Specific Conductivity, Water Analysis.

TABLE 2. SPECIFIC CONDUCTIVITY TESTS

Sample	A			B			C		
Probe	Cell VSO2	Cell VS2	Cell VS2O	Cell VSO2	Cell VS2	Cell VS2O	Cell VSO2	Cell VS2	Cell VS2O
1									
Cell Constant									
Sp. Cond. µmhos/cm									

DISSOLVED OXYGEN
DETERMINATION BY ELECTRONIC MEASUREMENT

I INTRODUCTION

A Electronic measurement of DO is attractive for several reasons:

1 Electronic methods are more readily adaptable for automated analysis, continuous recording, remote sensing or portability.

2 Application of electronic methods with membrane protection of sensors affords a high degree of interference control.

3 Versatility of the electronic system permits design for a particular measurement, situation or use.

4 Many more determinations per man-hour are possible with a minor expenditure of time for calibration.

B Electronic methods of analysis impose certain restrictions upon the analyst to insure that the response does, in fact, indicate the item sought.

1 The ease of reading the indicator tends to produce a false sense of security. Frequent and careful calibrations are essential to establish workability of the apparatus and validity of its response.

2 The use of electronic devices requires a greater degree of competence on the part of the analyst. Understanding of the behavior of oxygen must be supplemented by an understanding of the particular instrument and its behavior during use.

C Definitions

1 Electrochemistry - a branch of chemistry dealing with relationships between electrical and chemical changes.

2 Electronic measurements or electrometric procedures - procedures using the measurement of potential differences as an indicator of reactions taking place at an electrode or plate.

3 Reduction - any process in which one or more electrons are added to an atom or an ion, such as $O_2 + 2e \rightarrow 2O^{=}$ The oxygen has been reduced.

4 Oxidation - any process in which one or more electrons are removed from an atom or an ion, such as $Zn^o - 2e \rightarrow Zn^{+2}$. The zinc has been oxidized.

5 Oxidation - reduction reactions - in a strictly chemical reaction, reduction cannot occur unless an equivalent amount of some oxidizable substance has been oxidized. For example:

$2H_2 + O_2 \rightleftarrows 2H_2O$

$2H_2 - 4e \rightleftarrows 4H^{+1}$ hydrogen oxidized

$O_2 + 4e \rightleftarrows 2O^{-2}$ oxygen reduced

Chemical reduction of oxygen may also be accomplished by electrons supplied to a noble metal electrode by a battery or other energizer.

6 Anode - an electrode at which oxidation of some reactable substance occurs.

7 Cathode - an electrode at which reduction of some reactable substance occurs. For example in I. C. 3, the reduction of oxygen occurs at the cathode.

8 Electrochemical reaction - a reaction involving simultaneous conversion of chemical energy into electrical energy or the reverse. These conversions are

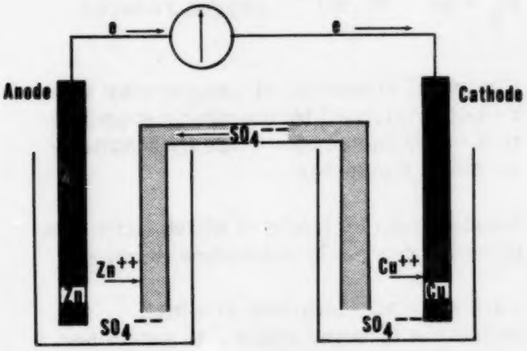
Note: Mention of Commercial Products and Manufacturers Does Not Imply Endorsement by the Environmental Protection Agency.

equivalent in terms of chemical and electrical energy and generally are reversible.

9 Electrolyte a solution, gel, or mixture capable of conducting electrical energy and serving as a reacting media for chemical changes. The electrolyte commonly contains an appropriate concentration of selected mobile ions to promote the desired reactions.

10 Electrochemical cell - a device consisting of an electrolyte in which 2 electrodes are immersed and connected via an external metallic conductor. The electrodes may be in separate compartments connected by tube containing electrolyte to complete the internal circuit.

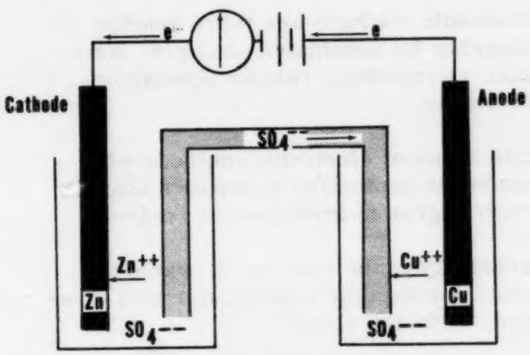
a Galvanic (or voltaic) cell - an electrochemical cell operated in such a way as to produce electrical energy from a chemical change, such as a battery (See Figure 1).



GALVANIC CELL

Figure 1

b Polarographic (electrolytic) cell - an electrochemical cell operated in such a way as to produce a chemical change from electrical energy (See Figure 2).



POLAROGRAPHIC CELL

Figure 2

D As indicated in I. C. 10 the sign of an electrode may change as a result of the operating mode. The conversion by the reactant of primary interest at a given electrode therefore designates terminology for that electrode and operating mode. In electronic oxygen analyzers, the electrode at which oxygen reduction occurs is designated the cathode.

E Each cell type has characteristic advantages and limitations. Both may be used effectively.

1 The galvanic cell depends upon measurement of electrical energy produced as a result of oxygen

reduction. If the oxygen content of the sample is negligible, the measured current is very low and indicator driving force is negligible, therefore response time is longer.

2 The polarographic cell uses a standing current to provide energy for oxygen reduction. The indicator response depends upon a change in the standing current as a result of electrons released during oxygen reduction. Indicator response time therefore is not dependent upon oxygen concentration.

3 Choice may depend upon availability, habit, accessories, or the situation. In each case it is necessary to use care and judgment both in selection and use for the objectives desired.

II ELECTRONIC MEASUREMENT OF DO

A Reduction of oxygen takes place in two steps as shown in the following equations:

1 $O_2 + 2H_2O + 2e \rightarrow H_2O_2 + 2OH^-$

2 $H_2O_2 + 2e^- \rightarrow 2OH^-$

Both equations require electron input to activate reduction of oxygen. The first reaction is more important for electronic DO measurement because it occurs at a potential (voltage) which is below that required to activate reduction of most interfering components (0.3 to 0.8 volts relative to the saturated calomel electrode - SCE). Interferences that may be reduced at or below that required for oxygen usually are present at lower concentrations in water or may be minimized by the use of a selective membrane or other means. When reduction occurs, a definite quantity of electrical energy is produced that is proportional to the quantity of reductant entering the reaction. Resulting current measurements thus are more specific for oxygen reduction.

B Most electronic measurements of oxygen are based upon one of two techniques for evaluating oxygen reduction in line with equation II.A.1. Both require activating energy, both produce a current proportional to the quantity of reacting reductant. The techniques differ in the means of supplying the activating potential; one employs a source of outside energy, the other uses spontaneous energy produced by the electrode pair.

1 The polarographic oxygen sensor relies upon an outside source of potential to activate oxygen reduction. Electron gain by oxygen changes the reference voltage.

a Traditionally, the dropping mercury electrode (DME) has been used for polarographic measurements. Good results have been obtained for DO using the DME but the difficulty of maintaining a constant mercury drop rate, temperature control, and freedom from turbulence makes it impractical for field use.

b Solid electrodes are attractive because greater surface area improves sensitivity. Poisoning of the solid surface electrodes is a recurrent problem. The use of selective membranes over noble metal electrodes has minimized but not eliminated electrode contamination. Feasibility has been improved sufficiently to make this type popular for regular use.

2 Galvanic oxygen electrodes consist of a decomposable anode and a noble metal cathode in a suitable electrolyte to produce activating energy for oxygen reduction (an air cell or battery). Lead is commonly used as the anode because its decomposition potential favors spontaneous reduction of oxygen. The process is continuous as long as lead and oxygen are in contact in the electrolyte and the electrical energy released at the cathode may be dissipated by an outside circuit. The anode may be conserved by limiting oxygen availability. Interrupting the outside circuit may produce erratic behavior for a time after reconnection. The resulting

current produced by oxygen reduction may be converted to oxygen concentration by use of a sensitivity coefficient obtained during calibration. Provision of a pulsed or interrupted signal makes it possible to amplify or control the signal and adjust it for direct reading in terms of oxygen concentration or to compensate for temperature effects.

III ELECTRONIC DO ANALYZER APPLICATION FACTORS

A Polarographic or galvanic DO instruments operate as a result of oxygen partial pressure at the sensor surface to produce a signal characteristic of oxygen reduced at the cathode of some electrode pair. This signal is conveyed to an indicating device with or without modification for sensitivity and temperature or other influences depending upon the instrument capabilities and intended use.

1 Many approaches and refinements have been used to improve workability, applicability, validity, stability and control of variables. Developments are continuing. It is possible to produce a device capable of meeting any reasonable situation, but situations differ.

2 Most commercial DO instruments are designed for use under specified conditions. Some are more versatile than others. Benefits are commonly reflected in the price. It is essential to determine the requirements of the measurement situation and objectives for use. Evaluation of a given instrument in terms of sensitivity, response time, portability, stability, service characteristics, degree of automation, and consistency are used for judgment on a cost/benefit basis to select the most acceptable unit.

B Variables Affecting Electronic DO Measurement

1 Temperature affects the solubility of oxygen, the magnitude of the resulting signal and the permeability of the protective membrane. A curve of oxygen solubility in water versus increasing temperature may be concave downward while a similar curve of sensor response versus temperature is concave upward. Increasing temperature decreases oxygen solubility and increases probe sensitivity and membrane permeability. Thermistor actuated compensation of probe response based upon a linear relationship or average of oxygen solubility and electrode sensitivity is not precisely correct as the maximum spread in curvature occurs at about 17° C with lower deviations from linearity above or below that temperature. If the instrument is calibrated at a temperature within + or - 5° C of working temperature, the compensated readout is likely to be within 2% of the real value. Depending upon probe geometry, the laboratory sensor may require 4 to 6% correction of signal per ° C change in liquid temperature.

2 Increasing pressure tends to increase electrode response by compression and contact effects upon the electrolyte, dissolved gases and electrode surfaces. As long as entrained gases are not contained in the electrolyte or under the membrane, these effects are negligible.

Inclusion of entrained gases results in erratic response that increases with depth of immersion.

3 Electrode sensitivity changes occur as a result of the nature and concentration of contaminants at the electrode surfaces and possible physical chemical or electronic side reactions produced. These may take the form of a physical barrier, internal short, high residual current, or chemical changes in the metal surface. The membrane is intended to allow dissolved gas penetration but to exclude passage of ions or particulates. Apparently some ions or materials producing extraneous ions within the electrode vicinity are able to pass in limited amounts which

become significant in time. Dissolved gases include 1) oxygen, 2) nitrogen, 3) carbon dioxide, 4) hydrogen sulfide, and certain others. Item 4 is likely to be a major problem. Item 3 may produce deposits in alkaline media; most electrolytes are alkaline or tend to become so in line with reaction II.A.1. The usable life of the sensor varies with the type of electrode system, surface area, amount of electrolyte and type, membrane characteristics, nature of the samples to which the system is exposed and the length of exposure. For example, galvanic electrodes used in activated sludge units showed that the time between cleanup was 4 to 6 months for electrodes used for intermittent daily checks of effluent DO; continuous use in the mixed liquor required electrode cleanup in 2 to 4 weeks. Each electrometric cell configuration and operating mode has its own response characteristics. Some are more stable than others. It is necessary to check calibration frequency required under conditions of use as none of them will maintain uniform response indefinitely. Calibration before and after daily use is advisable.

4 Electrolytes may consist of solutions or gels of ionizable materials such as acids, alkalies or salts. Bicarbonates, KC1 and KI are frequently used. The electrolyte is the transfer and reaction media, hence, it necessarily becomes contaminated before damage to the electrode surface may occur. Electrolyte concentration, nature, amount and quality affect response time, sensitivity, stability, and specificity of the sensor system. Generally a small quantity of electrolyte gives a shorter response time and higher sensitivity but also may be affected to a greater extent by a given quantity of contaminating substances.

5 Membranes may consist of teflon, polyethylene, rubber, and certain other polymeric films. Thickness may vary from 0.013 to 0.076 mm (0.5 to 3 mils). A thinner membrane will decrease response time and increase sensitivity but is less selective and may be ruptured more easily. The choice of material and its uniformity affects response time, selectivity and durability. The area of the membrane and its permeability are directly related to the quantity of transported materials that may produce a signal. The permeability of the membrane material is related to temperature and to residues accummulated on the membrane surface or interior. A cloudy membrane usually indicates deposition and more or less loss of signal.

6 Test media characteristics control the interval of usable life between cleaning and rejuvenation for any type of electrode. More frequent cleanup is essential in low quality waters than for high quality waters. Reduced sulfur compounds are among the more troublesome contaminants. Salinity affects the partial pressure of oxygen at any given temperature. This effect is small compared to most other variables but is significant if salinity changes by more than 500 mg/L.

7 Agitation of the sample in the vicinity of the electrode is important because DO is reduced at the cathode. Under quiescent conditions a gradient in dissolved oxygen content would be established on the sample side of the membrane as well as on the electrode side, resulting in atypical response. The sample should be agitated sufficiently to deliver a representative portion of the main body of the liquid to the outer face of the membrane. It is commonly observed that no agitation will result in a very low or negigible response after a short period of time. Increasing agitation will cause the response to rise gradually until some minimum liquid velocity is reached that will not cause a further increase in response with increased mixing energy. It is important to check mixing velocity to reach a stable high signal that is independent of a reasonable change in sample mixing. Excessive

mixing may create a vortex and expose the sensing surface to air rather than sample liquid. This should be avoided. A linear liquid velocity of about 30.5 cm/sec (1 ft/sec) at the sensing surface is usually adequate.

8 DO sensor response represents a potential or current signal in the milli-volt or milli-amp range in a high resistance system. A high quality electronic instrument is essential to maintain a usable signal-to-noise ratio. Some of the more common difficulties include:

a Variable line voltage or low batteries in amplifier power circuits.

b Substandard or unsteady amplifier or resistor components.

c Undependable contacts or junctions in the sensor, connecting cables, or instrument control circuits.

d Inadequately shielded electronic components.

e Excessive exposure to moisture, fumes or chemicals in the wrong places lead to stray currents, internal shorts or other malfunction.

C Desirable Features in a Portable DO Analyzer

1 The unit should include steady state performance electronic and indicating components in a convenient but sturdy package that is small enough to carry.

2 There should be provisions for addition of special accessories such as bottle or field sensors, agitators, recorders, line extensions, if needed for specific requirements. Such additions should be readily attachable and detachable and maintain good working characteristics.

3 The instrument should include a sensitivity adjustment which upon calibration will provide for direct reading in terms of mg of DO/L.

4 Temperature compensation and temperature readout should be incorporated.

5 Plug in contacts should be positive, sturdy, readily cleanable and situated to minimize contamination. Water seals should be provided where necessary.

6 The sensor should be suitably designed for the purpose intended in terms of sensitivity, response, stability, and protection during use. It should be easy to clean, and reassemble for use with a minimum loss of service time.

7 Switches, connecting plugs, and contacts preferably should be located on or in the instrument box rather than at the "wet" end of the line near the sensor. Connecting cables should be multiple strand to minimize separate lines. Calibration controls should be convenient but designed so that it is not likely that they will be inadvertently shifted during use.

8 Agitator accessories for bottle use impose special problems because they should be small, self contained, and readily detachable but sturdy enough to give positive agitation and electrical continuity in a wet zone.

9 Major load batteries should be rechargeable or readily replaceable. Line operation should be feasible wherever possible.

10 Service and replacement parts availability are a primary consideration. Drawings, parts identification and trouble shooting memos should be incorporated with applicable operating instructions in the instrument manual in an informative organized form.

D Sensor and Instrument Calibration

The instrument box is likely to have some form of check to verify electronics, battery or other power supply conditions for use. The sensor commonly is not included in this check. A known reference sample used with the instrument in an operating mode is the best available method to compensate for sensor variables under use conditions. It is advisable to calibrate before and after daily use under test conditions. Severe conditions, changes in conditions, or possible damage call for calibrations during the use period. The readout scale is likely to be labeled - calibration is the basis for this label.

The following procedure is recommended:

1 Turn the instrument on and allow it to reach a stable condition. Perform the recommended instrument check as outlined in the operating manual.

2 The instrument check usually includes an electronic zero correction. Check each instrument against the readout scale with the sensor immersed in an agitated solution of sodium sulfite containing sufficient cobalt chloride to catalyze the reaction of sulfite and oxygen. The indicator should stabilize on the zero reading. If it does not, it may be the result of residual or stray currents, internal shorting in the electrode, or membrane rupture. Minor adjustments may be made using the indicator rather than the electronic controls. Serious imbalance requires electrode reconditioning if the electronic check is O.K. Sulfite must be carefully rinsed from the sensor until the readout stabilizes to prevent carry over to the next sample.

3 Fill two DO bottles with replicate samples of clarified water similar to that to be tested. This water should not contain significant test interferences.

4 Determine the DO in one by the azide modification of the iodometric titration.

5 Insert a magnetic stirrer in the other bottle or use a probe agitator. Start agitation after insertion of the sensor assembly and note the point of stabilization.

a Adjust the instrument calibration control if necessary to compare with the titrated DO.

b If sensitivity adjustment is not possible, note the instrument stabilization point and designate it as ua. A sensitivity coefficient, ϕ is equal to $\frac{\mu a}{DO}$ where DO is the titrated value for the sample on which ua was obtained. An unknown DO then becomes $DO = \frac{\mu a}{\phi}$. This factor is applicable as long as the sensitivity does not change.

6 Objectives of the test program and the type of instrument influence calibration requirements. Precise work may require calibration at 3 points in the DO range of interest instead of at zero and high range DO. One calibration point frequently may be adequate.

Calibration of a DO sensor in air is a quick test for possible changes in sensor response. The difference in oxygen content of air and of water is too large for air calibration to be satisfactory for precise calibration for use in water.

IV This section reviews characteristics of several sample laboratory instruments. Mention of a specific instrument does not imply USEPA endorsement or recommendation. No attempt has been made to include all the available instruments; those described are used to indicate the approach used at one stage of development which may or may not represent the current available model.

A The electrode described by Carrit and Kanwisher (1) is illustrated in Figure 3. This electrode was an early example of those using a membrane. The anode was a silver - silver oxide reference cell with a platinum disc cathode (1-3 cm diameter). The salt bridge consisted of N/2 KCl and

KOH. The polyethylene membrane was held in place by a retaining ring. An applied current was used in a polarographic mode. Temperature effects were relatively large. Thermistor correction was studied but not integrated with early models.

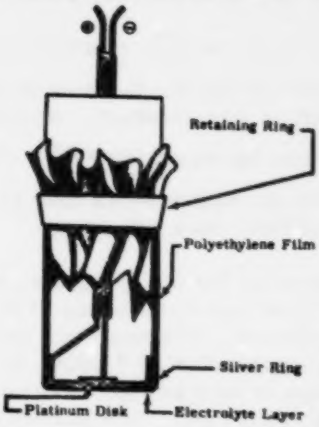

Retaining Ring
Polyethylene Film
Silver Ring
Platinum Disk
Electrolyte Layer

Figure 3

B The Beckman oxygen electrode is another illustration of a polarographic DO sensor (Figure 4). It consists of a gold cathode, a silver anode, an electrolytic gel containing KCl, covered by a teflon membrane. The instrument has a temperature readout and compensating thermistor, a source polarizing current, amplifier with signal adjustment and a readout DO scale with recorder contacts.

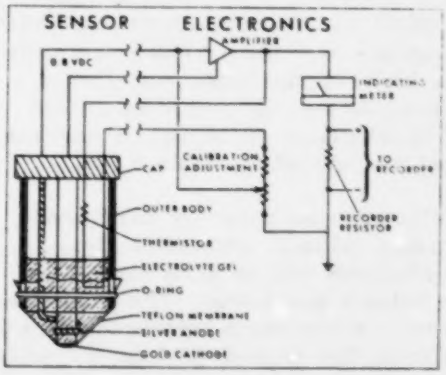

SENSOR
ELECTRONICS
AMPLIFIER
0.8 VDC
INDICATING METER
CALIBRATION ADJUSTMENT
TO RECORDER
RECORDER RESISTOR
CAP
OUTER BODY
THERMISTOR
ELECTROLYTE GEL
O. RING
TEFLON MEMBRANE
SILVER ANODE
GOLD CATHODE

Figure 4. THE BECKMAN OXYGEN SENSOR

C The YSI Model 51 (3) is illustrated in Figure 5. This is another form of polarographic DO analyzer. The cell consists of a silver anode coil, a gold ring cathode and a KCl electrolyte with a teflon membrane. The instrument has a sensitivity adjustment, temperature and DO readout. The model 51 A has temperature compensation via manual preset dial. A field probe and bottle probe are available.

YSI Model 51 DO Sensor

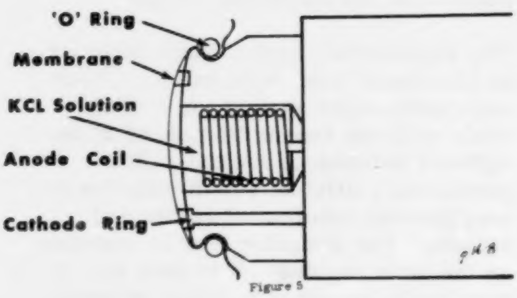

'O' Ring
Membrane
KCL Solution
Anode Coil
Cathode Ring

Figure 5

D The Model 54 YSI DO analyzer (4) is based upon the same electrode configuration but modified to include automatic temperature compensation, DO readout, and recorder jacks. A motorized agitator bottle probe is available for the Model 54 (Figure 6).

YSI Model 54 Agitator Probe

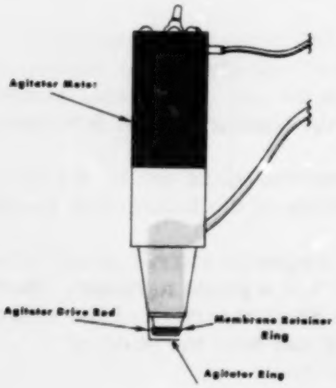

Agitator Motor
Agitator Drive Rod
Membrane Retainer Ring
Agitator Ring

Figure 6

E The Galvanic Cell Oxygen Analyzer (7, 8) employs an indicator for proportional DO signal but does not include thermistor compensation or signal adjustment. Temperature readout is provided. The sensor includes a lead anode ring, and a silver cathode with KOH electrolyte (4 molar) covered by a membrane film (Figure 7).

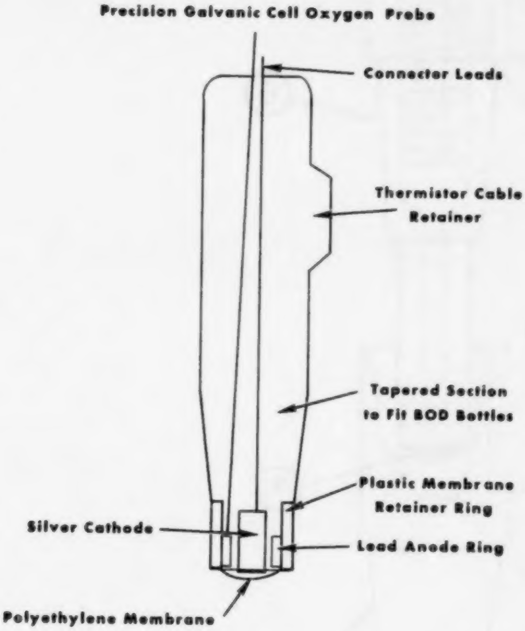

Precision Galvanic Cell Oxygen Probe
Connector Leads
Thermistor Cable Retainer
Tapered Section to Fit BOD Bottles
Plastic Membrane Retainer Ring
Silver Cathode
Lead Anode Ring
Polyethylene Membrane

Figure 7

F The Weston and Stack Model 300 DO Analyzer (8) has a galvanic type sensor with a pulsed current amplifier adjustment to provide for signal and temperature compensation. DO and temperature readout is provided. The main power supply is a rechargeable battery. The sensor (Figure 8) consists of a lead anode coil recessed in the electrolyte cavity (50% KI) with a platinum cathode in the tip. The sensor is covered with a teflon membrane. Membrane retention by rubber band or by a plastic retention ring may be used for the bottle agitator or depth sampler respectively. The thermistor and agitator are mounted in a sleeve that also provides protection for the membrane.

G The EIL Model 15 A sensor is illustrated in Figure 9. This is a galvanic cell with thermistor activated temperature compensation and readout. Signal adjustment is provided. The illustration shows an expanded scheme of the electrode which when assembled compresses into a sensor approximating 1.59 cm diameter and 10.2 cm length exclusive of the enlargement at the upper end. The anode consists of compressed lead shot in a replaceable capsule (later models used fine lead wire coils), a perforated silver cathode sleeve around the lead is covered by a membrane film. The electrolyte is saturated potassium bicarbonate. The large area of lead surface, silver and membrane provides a current response of 200 to 300 microamperes in oxygen saturated water at 20° C for periods of up to 100 days use (8). The larger electrode displacement favors a scheme described by Eden (9) for successive DO readings for BOD purposes.

V Table 1 summarizes major characteristics of the sample DO analyzers described in Section IV. It must be noted that an ingenious analyst may adapt any one of these for special purposes on a do-it-yourself program. The sample instruments are mainly designed for laboratory or portable field use. Those designed for field monitoring purposes may include similar designs or alternate designs generally employing larger anode, cathode, and electrolyte capacity to approach better response stability with some sacrifice in response time and sensitivity. The electronic controls, recording, telemetering, and accessory apparatus generally are semi-permanent installations of a complex nature.

ACKNOWLEDGMENTS:

This outline contains certain materials from previous outlines by D. G. Ballinger, N. C. Malof, and J. W. Mandia. Additional information was provided by C. R. Hirth, C. N. Shadix, D. F. Krawczyk, J. Woods, and others.

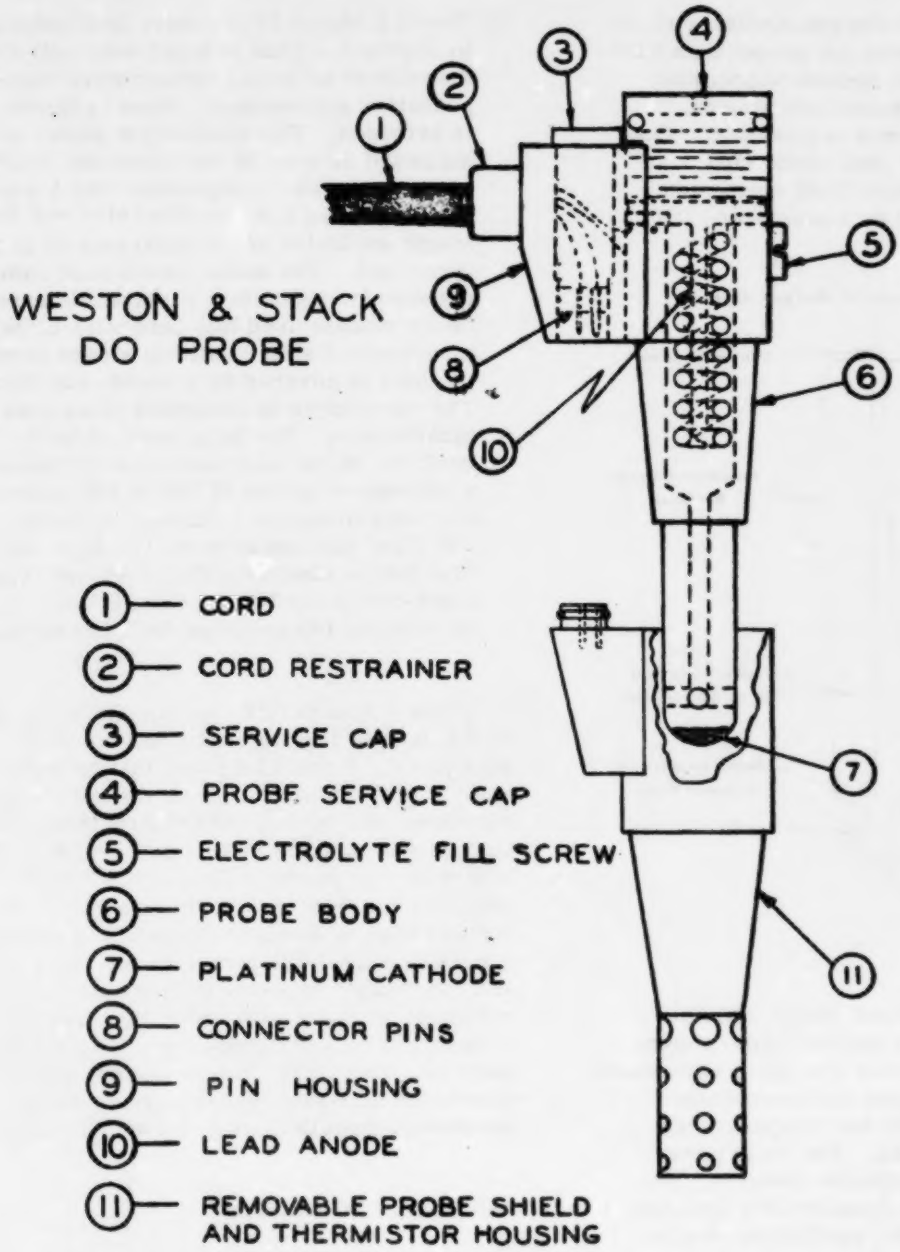
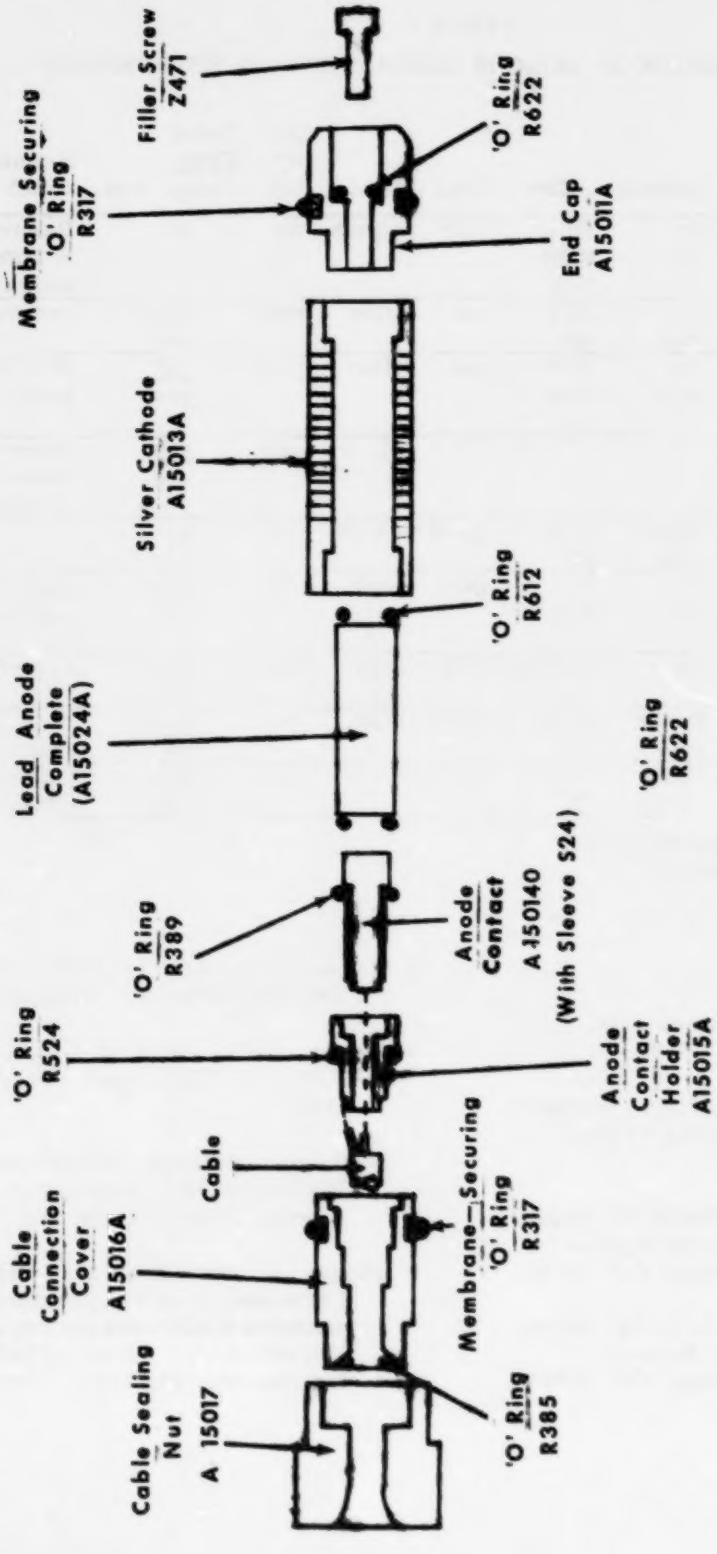

WESTON & STACK
DO PROBE
1
2
3
4
5
6
7
8
9
10
11
① — CORD
② — CORD RESTRAINER
③ — SERVICE CAP
④ — PROBE SERVICE CAP
⑤ — ELECTROLYTE FILL SCREW
⑥ — PROBE BODY
⑦ — PLATINUM CATHODE
⑧ — CONNECTOR PINS
⑨ — PIN HOUSING
⑩ — LEAD ANODE
⑪ — REMOVABLE PROBE SHIELD AND THERMISTOR HOUSING

Figure 8

Model A15A ELECTRODE COMPONENT PARTS


Cable Sealing Nut
A 15017
'O' Ring R385
Cable Connection Cover A15016A
Membrane Securing 'O' Ring R317
Cable
'O' Ring R524
Anode Contact Holder A15015A
'O' Ring R389
Anode Contact
A 150140
(With Sleeve S24)
Lead Anode Complete (A15024A)
'O' Ring R612
'O' Ring R622
Silver Cathode A15013A
Membrane Securing 'O' Ring R317
End Cap A15011A
Filler Screw Z471
'O' Ring R622

Note: Red wire of cable connects to Anode Contact Holder

Black wire of cable connects to Anode Contact

Membrane not shown E. I. L. part number T22

Figure 9

TABLE 1

CHARACTERISTICS OF VARIOUS LABORATORY DO INSTRUMENTS

	Anode	Cathode	Elec	Type	Membr	DO Sig. Adj.	Temp. Comp. Temp. Rdg.	Accessories for which designed
Carrit & Kanwisher	silver-silver ox. ring	Pt disc	KCl KOH N/2	pol*	polyeth	no	no	Recording temp. & signal adj. self assembled
Beckman	Aq ring	Au disc	KCl gel	pol	teflon	yes	yes yes	recording
Yellow Springs 51	Ag coil	Au ring	KCl soln sat.	pol	teflon	yes	no* yes	field and bottle probe
Yellow Springs 54	"	"	"	"	"	yes	yes yes	recording field bottle & agitator probes
Precision Sci	Pb ring	silver disc	KOH 4N	galv**	polyeth	no	no yes	
Weston & Stack 300	Pb coil	Pt disc	KI 40%	galv	teflon	yes	yes yes	agit. probe depth sampler
EIL	Pb	Ag	$KHCO_3$	galv	teflon	yes	yes yes	recording
Delta 75	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes no	field bottle & agitator probe
Delta 85	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes yes	field bottle & agitator probe

*Pol - Polarographic (or amperometric)
**Galv - Galvanic (or voltametric)

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This outline was prepared by F. J. Ludzack, former Chemist, National Training Center, OWPO, USEPA, Cincinnati, OH 45268 and Nate Malof, Chemist, USEPA, OWPO, National Field Investigations Center, Cincinnati, OH

Descriptors: Chemical Analysis, Dissolved Oxygen, Dissolved Oxygen Analyzers, Instrumentation, On-Site Tests, Water Analysis, Analysis, Wastewater, Oxygen

LABORATORY PROCEDURE FOR DISSOLVED OXYGEN
Winkler Method-Azide Modification

I APPLICABILITY

A The azide modification is used for most wastewaters and streams which contain nitrate nitrogen and not more than 1 mg of ferrous iron/L. If 1 mL 40% KF solution is added before acidifying the sample and there is no delay in titration, the method is also applicable in the presence of 100-200 mg ferric iron/L.

B Reducing and oxidizing materials should be absent.

C Other materials which interfere with the azide modification are: sulfite, thiosulfate, appreciable quantities of free chlorine or hypochlorite, high suspended solids, organic substances readily oxidized in a highly alkaline medium, organic substances readily oxidized by iodine in an acid medium, untreated domestic sewage, biological flocs, and color which may interfere with endpoint detection. A dissolved oxygen meter should be used when these materials are present in the sample.

II REAGENTS

Distilled water is to be used for the preparation of all solutions.

A Manganous Sulfate Solution

Dissolve 480 g $MnSO_4 \cdot 4H_2O$ (or 400 g $MnSO_4 \cdot 2H_2O$, or 364 g $MnSO_4 \cdot H_2O$) in water and dilute to 1 liter.

B Alkaline-Iodide-Azide Solution

Dissolve 500 g sodium hydroxide (or 700 g potassium hydroxide) and 135 g sodium iodide (or 150 g potassium iodide) in water and dilute to 1 liter. To this solution add 10 g of sodium azide dissolved in 40 mL water.

C Sulfuric Acid, Conc.

The strength of this acid is 36 N.

D Starch Solution

Prepare an emulsion of 10 g of soluble starch in a mortar or beaker with a small quantity of water. Pour this emulsion into 1 liter of boiling water, allow to boil a few minutes, and let settle overnight. Use the clear supernate. This solution may be preserved by the addition of 5 mL per liter of chloroform and storage in a refrigerator at 10°C.

E Sodium Thiosulfate Stock Solution 0.75 N

Dissolve 186.15 g $Na_2S_2O_3 \cdot 5H_2O$ in boiled and cooled water and dilute to 1 liter. Preserve by adding 5 mL chloroform.

F Sodium Thiosulfate Standard Titrant 0.0375N

Dilute 50.0 mL of stock solution to 1 liter. Preserve by adding 5 mL of chloroform.

G Potassium Biiodate Solution 0.0375N

Dry about 5 g of $KH(IO_3)_2$ at 103°C for two hours and cool in a desiccator. Dissolve 4.873 g of the solid in water and dilute to 1 liter. Dilute 250 mL of this solution to 1 liter.

H Sulfuric Acid Solution 10%

Add 10 mL of conc sulfuric acid to 90 mL of water. Mix thoroughly and cool.

I Potassium Iodide Crystals

III STANDARDIZATION OF THE TITRANT

A Dissolve 1-3 g of potassium iodide in 100-150 mL of water.

B Add 10 mL of 10% sulfuric acid and mix.

C Pipet in 20 mL of the 0.0375N potassium biiodate and mix. Place in the dark for 5 minutes.

D Titrate with the 0.0375N sodium thiosulfate standard titrant to the appearance of a pale yellow color.

Mix the solution thoroughly during the titration.

E Add 1-2 mL of starch solution and mix. The solution is now blue in color.

F Continue the addition of the titrant, with thorough mixing, until the solution turns colorless.

G Record the mL of titrant used.

H Calculate the N of the sodium thiosulfate standard titrant. It will be approximately 0.0375.

$$N = \frac{(\text{mL} \times \text{N}) \text{ of the biiodate}}{\text{mL of titrant}}$$

$$= \frac{20.0 \times 0.0375}{\text{mL of titrant}}$$

$$= \frac{0.75}{\text{mL of titrant}}$$

IV PROCEDURE

A Addition of Reagents

1 Manganous sulfate and alkaline iodide-azide

To a full BOD bottle (300 mL + 3 mL), add 2 mL manganous sulfate solution and 2 mL alkaline-iodide azide reagent with the tip of each pipette below the surface of the liquid.

2 Stopper the bottle without causing formation of an air bubble.

3 Rinse under running water.

4 Mix well by inverting 4-5 times.

5 Allow the precipitate to settle until there is clear liquid above the floc.

6 Repeat the inverting and allow to settle until about 200 mL of clear liquid has formed above the floc.

7 Add 2 mL conc. Sulfuric acid with the tip of the pipette above the surface of the liquid.

8 Stopper the bottle without causing formation of an air bubble.

9 Rinse under running water.

10 Mix by inverting several times to dissolve the precipitate.

11 Pour contents of bottle into a wide-mouth 500 mL Erlenmeyer flask.

B TITRATION

1 Titrate with 0.0375N thiosulfate to a pale yellow color.

2 Add 1-2 mL starch solution and mix.

3 Continue the addition of the titrant, with thorough mixing, until the solution turns colorless.

4 Record the ml of titrant used.

C CALCULATION

$$\text{mg DO/L} = \frac{\text{mL titrant} \times \text{N titrant} \times 8 \times 1000}{\text{mL sample}}$$

If the N of the titrant exactly = 0.0375,

$$\text{mg DO/L} = \frac{\text{mL titrant} \times 0.0375 \times 8 \times 1000}{300}$$

$$= \text{mL titrant} \times 1$$

$$= \text{mL titrant}$$

REFERENCE

Methods for Chemical Analysis of Water & Wastes, U.S. Environmental Protection Agency, Environmental Monitoring & Support Laboratory, Cincinnati, Ohio 45268, 1974

This outline was prepared by C. R. Feldmann, Chemist, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Analytical Techniques, Chemical Analysis, Dissolved Oxygen, Laboratory Tests, Oxygen, Water Analysis

DATA SHEET

mL of titrant = ____________

N of titrant = ____________

mL of sample = ____________

$$\text{mg DO/L} = \frac{\text{mL titrant} \times \text{N titrant} \times 8 \times 1000}{\text{mL sample}}$$

$$= \frac{\quad \times \qquad \times 8 \times 1000}{\qquad\qquad}$$

= ____________

=

DISSOLVED OXYGEN
Factors Affecting DO Concentration in Water

I The Dissolved Oxygen determination is a very important water quality criteria for many reasons:

A Oxygen is an essential nutrient for all living organisms. Dissolved oxygen is essential for survival of aerobic organisms and permits facultative organisms to metabolize more effectively. Many desirable varieties of macro or micro organisms cannot survive at dissolved oxygen concentrations below certain minimum values. These values vary with the type of organisms, stage in their life history, activity, and other factors.

B Dissolved oxygen levels may be used as an indicator of pollution by oxygen demanding wastes. Low DO concentrations are likely to be associated with low quality waters.

C The presence of dissolved oxygen prevents or minimizes the onset of putrefactive decomposition and the production of objectionable amounts of malodorous sulfides, mercaptans, amines, etc.

D Dissolved oxygen is essential for terminal stabilization wastewaters. High DO concentrations are normally associated with good quality water.

E Dissolved oxygen changes with respect to time, depth or section of a water mass are useful to indicate the degree of stability or mixing characteristics of that situation.

F The BOD or other respirometric test methods for water quality are commonly based upon the difference between an initial and final DO determination for a given sample time interval and condition. These measurements are useful to indicate:

1 The rate of biochemical activity in terms of oxygen demand for a given sample and conditions.

2 The degree of acceptability (a bioassay technique) for biochemical stabilization of a given microbiota in response to food, inhibitory agents or test conditions.

3 The degree of instability of a water mass on the basis of test sample DO changes over an extended interval of time.

4 Permissible load variations in surface water or treatment units in terms of DO depletion versus time, concentration, or ratio of food to organism mass, solids, or volume ratios.

5 Oxygenation requirements necessary to meet the oxygen demand in treatment units or surface water situations.

G Minimum allowable DO concentrations are specified in all Water Quality standards.

II FACTORS AFFECTING THE DO CONCENTRATION IN WATER

A Physical Factors:

1 DO solubility in water for an air/water system is limited to about 9 mg DO/liter of water at 20°C. This amounts to about 0.0009% as compared to 21% by weight of oxygen in air.

2 Transfer of oxygen from air to water is limited by the interface area, the oxygen deficit, partial pressure, the conditions at the

interface area, mixing phenomena and other items.

Certain factors tend to confuse reoxygenation mechanisms of water aeration:

a The transfer of oxygen in air to dissolved molecular oxygen in water has two principal variables:

1) Area of the air-water interface.

2) Dispersion of the oxygen-saturated water at the interface into the body liquid.

The first depends upon the surface area of the air bubbles in the water or water drops in the air; the second depends upon the mixing energy in the liquid. If diffusors are placed in a line along the wall, dead spots may develop in the core. Different diffusor placement or mixing energy may improve oxygen transfer to the liquid two or threefold.

b Other variables in oxygen transfer include:

3) Oxygen deficit in the liquid.

4) Oxygen content of the gas phase.

5) Time.

If the first four variables are favorable, the process of water oxygenation is rapid until the liquid approaches saturation. Much more energy and time are required to increase oxygen saturation from about 95 to 100% than to increase oxygen saturation from 0 to about 95%. For example: An oxygen-depleted sample often will pick up significant DO during DO testing; changes are unlikely with a sample containing equilibrium amounts of DO.

c The limited solubility of oxygen in water compared to the oxygen content of air does not require the interchange of a large mass of oxygen per unit volume of water to change DO saturation. DO increases from zero to 50% saturation are common in passage over a weir.

d Aeration of dirty water is practiced for cleanup. Aeration of clean water results in washing the air and transferring fine particulates and gaseous contaminants to the liquid.

e One liter of air at room temperature contains about 230 mg of oxygen. An 18.9L carboy of water with 2 liters of gas space above the liquid has ample oxygen supply for equilibration of DO after storage for 2 or 3 days or shaking for 30 sec.

f Aeration tends toward evaporative cooling. Oxygen content becomes higher than saturation values at the test temperature, thus contributing to high blanks.

3 Oxygen solubility varies with the temperature of the water. Solubility at 10° C is about two times that at 30° C. Temperature often contributes to DO variations much greater than anticipated by

solubility. A cold water often has much more DO than the solubility limits at laboratory temperature. Standing during warmup commonly results in a loss of DO due to oxygen diffusion from the supersaturated sample. Samples warmer than laboratory temperature may decrease in volume due to the contraction of liquid as temperature is lowered. The full bottle at higher temperature will be partially full after shrinkage with air entrance around the stopper to replace the void. Oxygen in the air may be transferred to raise the sample DO. For example, a volumetric flask filled to the 1000 mL mark at 30° C will show a water level about 1.27 cm below the mark when the water temperature is reduced to 20° C. BOD dilutions should be adjusted to 20° C + or - 1 1/2° before filling and testing.

4 Water density varies with temperature with maximum water density at 4° C. Colder or warmer waters tend to promote stratification of water that interferes with distribution of DO because the higher density waters tend to seek the lower levels.

5 Oxygen diffusion in a water mass is relatively slow, hence vertical and lateral mixing are essential to maintain relatively uniform oxygen concentrations in a water mass.

6 Increasing salt concentration decreases oxygen solubility slightly but has a larger effect upon density stratification in a water mass.

7 The partial pressure of the oxygen in the gas above the water interface controls the oxygen solubility limits in the water. For example, the equilibrium concentration of oxygen in water is about 9 mg DO/l under one atmospheric pressure of

B Biological or Bio-Chemical Factors

1 Aquatic life requires oxygen for respiration to meet energy requirements for growth, reproduction, and motion. The net effect is to deplete oxygen resources in the water at a rate controlled by the type, activity, and mass of living materials present, the availability of food and favorability of conditions.

2 Algae, autotrophic bacteria, plants or other organisms capable of photosynthesis may use light energy to synthesize cell materials from mineralized nutrients with oxygen released in process.

a Photosynthesis occurs only under the influence of adequate light intensity.

b Respiration of alga is continuous.

c The dominant effect in terms of oxygen assets or liabilities of alga depends upon algal activity, numbers and light intensity. Gross algal productivity contributes to significant diurnal DO variations.

3 High rate deoxygenation commonly accompanies assimilation of readily available nutrients and conversion into cell mass or storage products. Deoxygenation due to cell mass respiration commonly occurs at some lower rate dependent upon the nature of the organisms present, the stage of decomposition and the degree of predation, lysis, mixing and regrowth. Relatively high

deoxygenation rates commonly are associated with significant growth or regrowth of organisms.

4 Micro-organisms tend to flocculate or agglomerate to form settleable masses particularly at limiting nutrient levels (after available nutrients have been assimilated or the number of organisms are large in proportion to available food).

a Resulting benthic deposits continue to respire as bed loads.

b Oxygen availability is limited because the deposit is physically removed from the source of surface oxygenation and algal activity usually is more favorable near the surface. Stratification is likely to limit oxygen transfer to the bed load vicinity.

c The bed load commonly is oxygen deficient and decomposes by anaerobic action.

d Anaerobic action commonly is characterized by a dominant hydrolytic or solubilizing action with relatively low rate growth of organisms.

e The net effect is to produce low molecular weight products from cell mass with a correspondingly large fraction of feedback of nutrients to the overlaying waters. These lysis products have the effect of a high rate or immediate oxygen demand upon mixture with oxygen containing waters.

f Turbulence favoring mixing of surface waters and benthic sediments commonly are associated with extremely rapid depletion of DO. Recurrent resuspension of thin benthic deposits may contribute to highly erratic DO patterns.

g Long term deposition areas commonly act like point sources of new pollution as a result of the feedback of nutrients from the deposit. Rate of reaction may be low for old materials but a low percentage of a large mass of unstable material may produce excessive oxygen demands.

C Tremendous DO variations are likely in a polluted water in reference to depth, cross section or time of day. More stabilized waters tend to show decreased DO variations although it is likely that natural deposits such as leaf mold will produce differences related to depth in stratified deep waters.

ACKNOWLEDGMENTS

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REFERENCE

1 Methods for Chemical Analysis of Water & Wastes, U.S. Environmental Protection Agency, Environmental Monitoring & Support Laboratory, Cincinnati, Ohio, 45268, 1974.

This outline was prepared by F. J. Ludzack, former Chemist, National Training Center, and revised by Charles R. Feldmann, Chemist, National Training & Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Aeration, Aerobic Conditions, Air-Water Interfaces, Anaerobic Conditions, Benthos, Biological Oxygen Demand, Dissolved Oxygen, Water Pollution, Water Quality

BIOCHEMICAL OXYGEN DEMAND TEST PROCEDURES

I OXYGEN DEMAND OF POLLUTED WATERS

Established practice includes common use of the BOD test as a tool for estimation of the bio-oxidizable fraction of surface waters or wastewaters discharged to them. Any index including a quantity per unit time such as the BOD_5 is a rate expression. The ultimate demand is more important than any one point on the progression. The results of a bottle test with minimum seeding and quiescent storage are not likely to be as high as those on the same influent in a mixing situation and abundant seed of secondary treatment or receiving waters. The BOD_5 is "a" fraction of total oxygen requirements.

A The particular technique used for BOD commonly is specified by State agencies and/or supervisors. They are required to interpret the results as obtained by laboratory testing. It is essential that the tester and the interpreters have a common understanding of what was done and how. It is highly advisable to maintain a given routine until all concerned agree upon a change.

1 Each particular routine has many undefinable factors. The particular routine is not as important as the consistency and capability with which the result was obtained.

2 This outline and Standard Methods(1) discusses several valid approaches for obtaining BOD results. Selection of "method" is not intended in this outline or in the EPA Methods Manual(2).

B The common 5-day incubation period for BOD testing is a result of tradition and cost. Initial lags are likely to be over and some unknown fraction of the total oxidizable mass has been satisfied after 5 days.

C A series of observations over a period of time makes it possible to estimate the total oxidizable mass and the fraction oxidized or remaining to be oxidized at any given time. The problem is to define the shape of the deoxygenation pattern and its limits. A fair estimate of the shape of the deoxygenation pattern is available by observations at 1, 2, or 3 days, 7 days and 14 days. Increased observations are desirable for more valid estimates of curve shape, rate of oxidation and total oxidizable mass or ultimate BOD.

D Increasing impoundment of surface waters and concurrent increases in complexity and stability of wastewater components emphasize the importance of long-term observation of BOD. The 5-day observation includes most of the readily oxidizable materials but a very small fraction of the stable components that are the main factors in impoundment behavior.

II DIRECT METHOD

A With relatively clean surface waters, the BOD may be determined by incubation of the undiluted sample for the prescribed time interval. This method is applicable only to those waters whose BOD is less than 8 mg/L and assumes the sample contains suitable organisms and accessory nutrients for optimum biological stabilization.

B Treated effluents, polluted surface waters, household and industrial wastewaters commonly require dilution to provide the excess oxygen required for the oxygen demand determination. General guidelines for dilution requirements for a given BOD range in terms of the percent of sample in BOD dilution water are:

For a 5-day BOD of
5-20 mg/L, use 25 to 100% sample

For a BOD of
20-100 mg/L, use 5 to 25% sample

For a BOD of
100-500 mg/L use 1 to 5% sample

For a BOD of
500-5000 mg/L, use 0.1 to 1.0% sample

III PROCEDURES

A Cylinder Dilution Technique

1 Using an assumed or estimated BOD value as a guide, calculate the factors for a range of dilutions to cover the desired depletions. Those dilutions ranging from a depletion of 2 mg/L and a residual of 1 mg/L are most reliable. At least three dilutions in duplicate should be used for an unknown sample.

2 Into a one-liter graduate cylinder (or larger container if necessary) measure accurately the required amount of mixed sample to give one liter of diluted waste. Fill to the one liter mark with dilution water. Carefully mix. The initial DO by calculation includes IDOD (VIII) a determined initial does not. Both are essential to estimate significance of IDOD. Entrapment of air bubbles during manipulation must be avoided.

3 Siphon the mixture from the cylinder into three 300 ml glass stoppered bottles, filling the bottles to overflowing.

4 Determine the DO concentration on one of the bottles by the appropriate Winkler modification and record as "Initial DO".

5 Incubate the two remaining bottles at 20°C in complete darkness. The incubated bottles should be water-sealed by immersion in a tray or by using a special water-seal bottle.

6 After 5 days of incubation, or other desired interval, determine the DO on the bottles. Average the DO concentration of the duplicates and report as "Final DO".

B Direct Dilution Technique

1 It may be more convenient to make the dilution directly in sample bottles of known capacity. A measured volume of sample may be added (as indicated in A-1) above, and the bottle filled with dilution water to make the desired sample concentration for incubation. In this case, the sample must be precisely measured, the bottle carefully filled, but not overfilled, and the bottle volumes comparable and known. Precision is likely to be poorer than for cylinder dilution.

2 Continue the procedure as in A-4, 5, and 6 above.

C Seeded Cylinder Dilution Technique

1 Many wastewaters may be partially or completely sterile as a result of chlorination, effects of other toxic chemicals, heat, unfavorable pH or other factors detrimental to biological activity. Validity of the BOD result depends upon the presence of organisms capable of prompt and effective biodegradation and favorable conditions during the particular test. Correction of the cause resulting in sterilization must be corrected by adjustment, dilution, etc., prior to reinoculation to achieve meaningful BOD data. Receiving water, biologically treated effluents, and soil suspensions are a good source of organisms likely to be adapted for stabilization of wastewaters. Untreated wastewaters provide numerous organisms but are likely to contain nutrients contributing to excessive seed corrections and may require appreciable time for adaptation before test waste oxidation becomes significant.

2 The amount of added inoculant must be determined by trial. The concentration added should initiate biochemical activity promptly but should not exert enough oxygen demand to unduly reduce the oxygen available for sample requirements.

3 Estimate the sample concentration desired in accordance with A-1 and C-2 above and add the sample aliquot to the dilution cylinder.

4 Add approximately half of the required amount of dilution water to the sample and mix. This is necessary to assure that the concentrated waste does not exert a toxic effect on the seed organisms.

5 Measure a suitable aliquot of seed into the bottle or cylinder and fill with dilution water. Mix the combined sample, seed and dilution water without excessive air entrainment.

6 Continue as in III-A steps 4, 5, and 6 above.

IV INTERPRETATION OF RESULTS

Standard Methods(1) includes a calculation section that is valid and concise. Preceding it are details of reagent preparation and procedures for the test. These will not be reprinted here. This section considers certain items that may cause concern about the validity of results unless they are carefully considered and controlled.

A The initial DO of the BOD test obviously should be high. The method of attaining a high DO can trap the analyst.

  1 Aeration of dilution water is the most commonly considered treatment. This technique does produce a high DO but it is a treacherous ally.

    a Dirty air passing through clean dilution water can produce clean air and dirty water. This is a simple air-washing operation. Filtering the entering air stream may remove brickbats and 2 x 4's, but filters tend to pass organic gases, fine aerosols, and particulates.

    b A stream of air passing through water tends to cool the water by evaporation 1 to 3°C below ambient temperature. The cooled liquid picks up more DO than it can hold at ambient temperature. The physical loss of oxygen may produce an erroneously high depletion value for a determined initial DO, or a low depletion on a calculated initial DO. Erroneous blanks are a particular concern. The dilution water temperature/DO shift is critical.

  2 Raising DO by allowing the sample to equilibrate in a cotton-plugged bottle for 2 or 3 days permits oxygenation with minimum air volume contact.

  3 Shaking a partially filled bottle for a few seconds also oxygenates with minimum opportunity of gas washing contamination, supersaturation, or temperature changes.

B Seeding always is a precarious procedure but a very necessary one at times. Often the application of seed corrections is a "_______if you do, _______if you don't" situation. Hopefully, seed corrections are small because each individual biological situation is a "universe" of its own.

  1 Unstable seeding materials such as fresh wastewater have "seed" organisms characteristic of their origin and history. Saprophytes resulting in surface water stabilization may be a small fraction of the population. Reactable oxygen-demanding components produce excessive demands upon test oxygen resources.

  2 A seed containing viable organisms at a lower energy state because of limited nutritional availability theoretically is the best available seed source. An organism population grown under similar conditions should be most effective for initiating biochemical activity as soon as the nutrient situation favors more activity. The population should not be stored too long because organism redistribution and die-out become limiting. This type of seed would most likely be found in a surface water or a treatment plant effluent with a history of receiving the particular material under consideration.

  3 Seed sources and amounts can only be evaluated by trial. Different seed sources and locations require checkout to determine the best available material from a standpoint of rapid initiation of activity, low correction, and predictable high oxygen depletion under test.

C Chlorination and BOD results fundamentally are incompatible. Chlorination objectives include disinfection as the number one goal. Chlorine is notoriously non-specific in organism effects. Chlorine acts like an oxidant in the DO determination. Test organisms are less suitable for activity than they were before chlorination. Nutrients may be less available after chlorination. Certainly the conditions are less suitable for biological response after chlorination. Dechlorination is feasible with respect to the oxidizing power of chlorine, but many organic chlorine compounds that do not show strong oxidizing action still have toxic effects on biologic response.

Numbers are obtainable after dechlorination and reseeding. The meaning of these numbers is obscure. At least two states (New York and New Jersey) specify BOD's before chlorination only.

V PRECISION OF THE BOD TEST

A The DO test precision often has been used to suggest precision of the BOD result.

DO precision is a relatively minor and controllable factor contributing to BOD results. Other factors such as organism suitability, members, adaptation and conditional variables are much more difficult to control or to evaluate.

B The Analytical Reference Service report on Water Oxygen Demand, July, 1960 (Sample type VII) included the results of seeded samples of glucose-glutamic acid BOD results from 34 agencies on 2, 3, 5 and 7 day incubations.

The relative geometric standard deviation (average) was 19% on 2% sample and 24% on 1% sample concentrations. Rate coefficients ranged from 0.10 to 0.27 with a median of 0.16 from 21 different laboratories that participated in rate studies.

VI ALTERNATE BOD TECHNIQUES

Reaeration methods are becoming increasingly popular in order to approach more nearly the actual waste concentration in the receiving water. It is common to obtain "sliding" BOD results related to the concentration of waste in a series of dilutions of the same sample. This may result from greater possibilities for toxic effects at higher concentrations, or to a different selection of organisms and change in oxidation characteristics at low concentrations of sample. The most reliable estimate of stream behavior is likely to be from that dilution closest to the wastewater dilution in the receiving water.

A Reaeration can be accomplished by the usual series techniques by dumping all of the remaining sealed bottles into a common container when the residual DO reaches about 1.0 mg/L. After reoxygenation, the remaining bottles are refilled and a new initial DO determined. Subsequent dissolved oxygen depletions are added incrementally as a summation of the total oxygen depletion from the start of the test. If necessary, the reaeration technique may be performed several times but at a sacrifice of double DO determinations for each day on which reaeration occurs.

B Special methods of reaeration have evolved to minimize the extra manipulation for reaeration of individual sample dilutions.

1 Elmore Method

A relatively large volume of the sample is stored in an unsealed bottle. Small bottles are withdrawn in sets of 5 or more, sealed, incubated, and the DO determined at appropriate intervals. When the DO concentration in the smaller bottles reaches 1.0 mg/L, a new set is withdrawn from the large unsealed bottle, after reoxygenation if necessary.

2 Orford Method

The deoxygenation is carried out in a large sealed jug from which samples for DO are withdrawn at appropriate intervals. To maintain the waste level and a sufficient DO in the jug, additional waste is added from a second open container. See diagram.

C Excess oxygen may be provided by oxygenation with commercial oxygen instead of with air to increase the initial oxygen content for incubation while limiting the number of dilutions or reaeration steps. When oxygen is used in place of air the oxygen saturation in water at 20°C is about 40 mg/L instead of 9mg/L. Limited results are available hence the analyst must verify his technique. The DO tends to decrease as soon as the bottle is opened hence, about 35 mg/L of oxygen content is the top of the practical working concentration. There has been no evidence that the biota is inhibited by the higher oxygen content with respect to BOD progression.

D Reaeration or Oxygenation Advantages and Limitations.

1 Reaeration expands the range of BOD results obtainable directly at field concentrations, but is not advisable for applications when the sample BOD exceeds 50 mg/L.

2 Dilution water problems are eliminated, to the extent that the stream sample may be tested without dilution.

3 Incubator storage space becomes a real problem for multiple sample routine.

VII Dissolved oxygen electrodes, polarographic and others, are feasible for use in BOD determinations, often making it possible to make an estimate of DO or BOD when sample interference prevents a valid Winkler DO determination.

Electronic probe DO makes it possible to determine many successive DO's at different time intervals on the same bottle with negligible sample loss. Reaeration or extended time series, therefore, are more feasible.

Another outline in this series describes response of reaerated BOD_5 with electronic DO probes.

A It is the responsibility of the analyst to evaluate:

1 Applicability of the specified technique and sample.

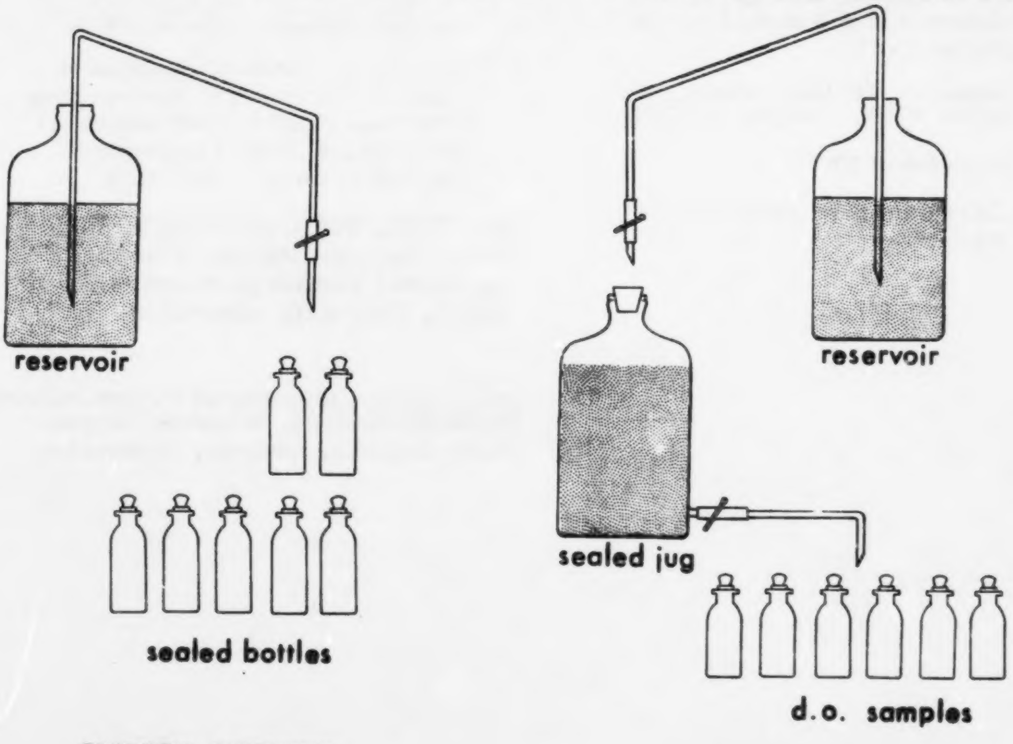
2 To determine requirements for mixing and possible thermal effects while mixing in terms of instrument response and biochemical reaction.

3 To evaluate long-term calibration or standardization and their effects upon precision and accuracy of the BOD result.

VIII IMMEDIATE DISSOLVED OXYGEN DEMAND (IDOD)

Immediate dissolved oxygen demand includes dissolved oxygen utilization requirements of substances such as ferrous iron, sulfite and sulfide which are susceptible to high rate chemical oxidation.

REAERATION METHODS FOR B.O.D. DETERMINATION



ELMORE METHOD

ORFORD METHOD

A The IDOD is an apparent response as indicated by a specified technique. Since DO titration is based upon iodine titration, any factor that causes I_2 response different from that produced by the reaction of KI and molecular oxygen confuses the IDOD determination.

B IDOD Determination

1 The IDOD determination includes the determination of DO on a sample and dilution water separately. A waste likely to have a significant IDOD is unlikely to show a DO.

2 According to mixing theory, it should be possible to calculate the DO of any definite mixture of the sample and dilution water from the DO of component parts and their proportion.

3 The same relative proportions of sample and dilution water should be mixed without air entrainment and the DO determined after the arbitrarily selected time of 15 minutes.

4 Any difference between the calculated initial DO as obtained in 2 above, and the DO determined in 3 above, may be designated as IDOD.

5 Sample aeration, DO interference, and other factors affect results for IDOD.

C Sample Calculation of IDOD

1 Sample DO checked and shown to be 0.0 mg/L

Dilution water DO found to be 8.2 mg/L

Assume a mixture of 9 parts of dilution water and 1 part (V/V) of sample.

Calculated DO =

$1 \times 0 = 0$

$9 \times 8.2 = 73.8$

10 parts of the mixture contain 73.8/10 or 7.4 mg DO/L. Note that mixing has reduced the DO concentration because the original amount is present in a larger package.

2 The mixture described above was held for 15 minutes and the DO determined was 4.3 mg/L.

$$\text{IDOD} = \text{DO}_{\text{calc}} - \text{DO}_{\text{detm}} \times \frac{100}{\%\ \text{sample used}}$$

$$= 7.4 - 4.3 \times 10$$

$$= 31 \text{ mg IDOD/L}$$

REFERENCES

1 Standard Methods, 14th ed, 1975.

2 Methods for Chemical Analysis of Water & Wastes, U.S. Environmental Protection Agency, Environmental Monitoring & Support Laboratory, Cincinnati, Ohio, 45268, 1974.

This outline was prepared by F. J. Ludzack, former Chemist, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Biochemical Oxygen Demand, Chemical Analysis, Dissolved Oxygen, Water Analysis, Analysis, Wastewater

BIOCHEMICAL OXYGEN DEMAND TEST
DILUTION TECHNIQUE

I GENERAL

A Standard Methods (1) lists three ways of diluting biochemical oxygen demand (BOD) samples: in a 1 or 2 liter graduated cylinder, in a bottle of known capacity (e.g., the BOD bottle), or in a volumetric flask for dilutions greater than 1:100, followed by final dilution in the incubation bottle.

B The dissolved oxygen (DO) determinations may be made using the azide modification of the Winkler procedure, or a DO meter.

II REAGENTS

A Distilled water - obtained from a block tin or all glass still; or use deionized water. It must contain no more than 0.01 mg of copper/L. It must be free of chlorine, chloramines, caustic alkalinity, organic material and acids. Aerate the water in one of three ways: loosely plug the container with cotton and store at 20°C for about 48 hours; shake 20°C water in a partially filled container; bubble clean compressed through 20°C water. Use distilled (but not necessarily aerated) water for the preparation of all solutions.

B Phosphate Buffer Solution - dissolve 8.5g potassium dihydrogen phosphate, KH_2PO_4, 21.75g dipotasium hydrogen phosphate, K_2HPO_4, 33.4g disodium hydrogen phosphate heptahydrate, $Na_2HPO_4 \cdot 7H_2O$, and 1.7g ammonium chloride, NH_4Cl, in about 500 mL of water and dilute to 1 L. The pH of this solution is 7.2 Discard it if any biological growth appears in the bottle.

C Magnesium Sulfate Solution - dissolve 22.5g magnesium sulfate heptahydrate, $MgSO_4 \cdot 7H_2O$, in water and dilute to 1 liter.

D Calcium Chloride Solution - dissolve 27.5g anhydrous calcium chloride, $CaCl_2$, in water and dilute to 1 liter.

E Ferric Chloride Solution - dissolve 0.25g ferric chloride, $FeCl_3$, in water and dilute to 1 liter.

F Dilution water - add 1 mL each, of solutions II B, II C, II D, and II E for each liter of distilled water (IIA). If the dilution water is to be stored, add the phosphate buffer (IIB) just before use.

G Seeded Dilution Water - the standard seed material is the supernatant liquid from domestic wastewater which has been allowed to settle for 24-36 hours at 20°C. Use an amount which will produce a seed correction of at least 0.6 mg/L. Add the seed to the dilution water (II F) on the day the dilution water is to be used.

H Sodium Sulfite Solution, 0.025N - dissolve 1.575g anhydrous sodium sulfite, Na_2SO_3, in water and dilute to 1 liter. Prepare this solution daily; it is unstable.

I Acetic Acid Solution 50% - slowly pour 50 mL acetic acid, $HC_2H_3O_2$, into 50 mL of water.

J Potassium Iodide Solution, 10% - dissolve 10g potassium iodide, KI, in 90 mL water.

K Sodium Hydroxide Solution, 1N -dissolve 4g sodium hydroxide, NaOH, in water and dilute to 100 mL.

L Sulfuric Acid Solution, 1N - slowly pour 2.8 mL of conc. sulfuric acid, H_2SO_4, into 98 mL of water.
Caution: heat will be generated.

M Powdered Starch Indicator - Thyodene is one brand name.

N Bromthymol Blue Indicator - or a pH meter.

III INTERFERENCES/PRETREATMENT

A Caustic Alkalinity or Acidity - this must be neutralized to a pH of about 7 with 1 N sulfuric acid or sodium hydroxide. Use a pH meter or bromthymol blue as an external indicator.

B Residual Chlorine Compounds - some residual chlorine will dissipate if the sample is allowed to stand for 1 or 2 hours. Higher residuals must be determined, and then neutralized. To a known volume of sample between 100 and 1000 mL, add 10 mL of acetic acid solution, 10 mL of potassium iodide solution, mix, and titrate to the disappearance of blue color with 0.025N sodium sulfite and using powdered starch indicator (or starch solution). Use a proportionate amount of the 0.025N sodium sulfite to dechlorinate the entire sample. (The portion of sample used above to determine the chlorine content of the sample should be discarded, and is not to be used for the BOD determination.) After 10-20 minutes, check a portion of the dechlorinated sample to make sure the dechlorination is complete.

C Other Toxic Substances - samples containing other toxic substances, e.g. metals in plating wastes, require special study and treatment.

D Supersaturation - if you suspect that the sample contains more than 9 mg of oxygen/L at 20°C, shake it vigorously in a large bottle or flask, or pass clean compressed air through the sample.

IV SUGGESTED SAMPLE DILUTIONS

Standard Methods (1) suggests the following sample dilutions. However, actual dilutions should be determined on the basis of experience, or information supplied with the sample.

Type of Waste	% Dilution
Strong Trade	0.1 - 1.0
Raw & Settled Sewage	1 - 5
Oxidized Effluents	5 - 25
Polluted River Waters	25 - 100

During the 5-day incubation period, at least 2 mg of oxygen/L must be consumed, and at least 1 mg of oxygen/L must remain at the end of the incubation period.

V PROCEDURE

The steps below represent one of several ways in which the BOD can be set up. For example purposes, assume the dilution water does not have to be seeded.

A Siphon 20°C high quality distilled water to the 1000 mL line in a graduated cylinder. Tilt the cylinder slightly and allow the water to run down the sides of the cylinder. If the siphon was "primed", with other water, "waste" about 100 mL before filling the cylinder.

B Add 1 mL of the calcium solution and mix with a plunger-type mixer.

C Add 1 mL of the magnesium solution and mix with a plunger-type mixer.

D Add 1 mL of the ferric solution and mix with a plunger-type mixer.

E Add 1 mL of the buffer solution and mix with a plunger-type mixer. (If the dilution water were to be seeded, it would be done at this point).

F Siphon about 250 mL of the dilution water into a 1 liter graduated cylinder. If more than 750 mL of sample are to be used, less than 250 mL of dilution water would, of course, be siphoned in initially. Use the same technique as in A above.

G Measure the amount of well mixed sample to be used. Use a graduated pipet for smaller sample volumes. If solids are present in the sample, the tip of the pipet may be cut off below the bottom graduation line. For larger sample volumes, use the appropriate size graduated cylinder.

H Add the sample to the cylinder containing the 250 mL of water. Allow the sample to run down the sides of the cylinder.

I Siphon in additional dilution water to the 1000 mL line, and mix with a plunger-type mixer. If other dilutions of the same sample, or other smaples, are being set up, be sure to rinse the mixer between uses.

J Siphon the dilution water-sample mixture into two BOD bottles. Hold the end of the siphon close to the bottom of the bottle, open the siphon slowly, and keep the tip of the siphon just above the surface of the surface of the liquid as the bottle fills. Allow a small amount of the mixture to overflow the bottle. If the siphon was "primed", "waste" about 100 mL before filling the bottles.

K Insert the stoppers into the BOD bottles with a slight twisting motion. Do not use so much force that an air bubble is created.

L Determine the initial DO (DOi) on one of the bottles within 15 minutes. Use the Winkler procedure, azide modification, or a DO meter.

M Water-seal the second bottle and incubate in the dark, at 20°C ± 1°C, for five days.

N Determine the final DO (DOf) on the second bottle. Use the same method as in L above. (Recall the restrictions noted at the end of section IV).

VI EXAMPLE CALCULATIONS

DO initial = DO i = 7.5 mg/L

DO final = DO f = 2.5 mg/L

100 mL = sample volume diluted in the 1 liter graduated cylinder = 10% dilution (0.1 as a decimal fraction)

$$\text{mg five-day BOD/l} = \frac{\text{DOi} - \text{DOf}}{\text{\% sample dilution expressed as a decimal}}$$

$$\text{mg five-day BOD/L} = \frac{7.5 - 2.5}{0.1}$$

$$= 50$$

VII SEED CORRECTION

A If you do seed the dilution water, a correction must be applied to the calculation in VI above.

B Do this by setting up another five-day BOD exactly as described above, except, use seed material instead of sample.

C In this case however, the five-day oxygen depletion must be 40-70%. (In the case of the sample it was a depletion of at least 2 mg/L with at least 1 mg/L remaining). Consequently, it may be necessary to set up several dilutions of the seed in order to get one with a 40-70% depletion.

D Example Seed Correction Calculation

Two hundred fifty mL of seed material are diluted to 1000 mL with dilution water.

$$\frac{250}{1000} \times 100 = 25\% \text{ seed material}$$

DO i = 7.0 mg/L

DO f = 3.0 mg/L

$$\text{Depletion} = 7.0 \text{ mg/L} - 3.0 \text{ mg/L}$$
$$= 4.0 \text{ mg/L}$$

$$\text{\% depletion} = \frac{4.0 \text{ mg/L}}{7.0 \text{ mg/L}} \times 100$$
$$= 56$$

Since the 25% seed dilution gave an oxygen depletion in the desired 40-70% range (56%), it can be used to calculate the seed correction.

E Example Seed Correction Calculation (Continued)

Assume that in preparing the dilution water (V A through V E), you added 2 ml of seed material to the graduated cylinder before adding dilution water to the 1000 mL line.

$\frac{2}{1000} \times 100 = 0.2\%$ seed material in the dilution water

F Example Seed Correction Calculation (Continued)

In the example calculation in VI, a 10% sample dilution was assumed.

If the BOD bottles contained 10% sample, they therefore contained 90% dilution water.

3 00 mL (volume of BOD bottles)
0.90 (% dilution water in the BOD bottles expressed as a decimal)

270.00 mL (volume of dilution water in the BOD bottles)

G Example Seed Correction Calculation (Continued)

270 mL (volume of dilution water in the BOD bottles)
0.002 (% seed material in the dilution water expressed as a decimal)
0.540 mL
0.540 mL (volume of seed material in the BOD bottles)

H Example Seed Correction Calculation (Continued)

$\frac{0.54}{3\,00} \times 100 = 0.18\%$ seed material in the BOD bottles

I You now have all the data you need to calculate the seed correction.

$$\text{mg five-day BOD/L} = \frac{(\text{DOi} - \text{DOf})\text{ of sample} - [(\text{DOi} - \text{DOf})\text{ of seed material} \times \text{factor}]}{\%\text{ of sample expressed as a decimal}}$$

DOi of sample = 7.5 mg/L (from VI above)

DOf of sample = 2.5 mg/L (from VI above)

DOi of seed material = 7.0 mg/L (from VII D above)

DOf of seed material = 3.0 mg/L (from VII D above)

% of seed in the sample BOD bottles = 0.18 (from VII H above)

% of seed in the seed BOD bottles = 25 (from VII D above)

% of sample expressed as a decimal fraction = 0.1 (from VI above)

$$\text{factor} = \frac{\%\text{ of seed in the sample BOD bottles}}{\%\text{ of seed in the seed BOD bottles}}$$

$$= \frac{0.18}{25}$$

$$= 0.0072$$

Finally, $\text{mg five-day BOD/L} = (7.5 - 2.5) - [(7.0 - 3.0) \times 0.0072]$

$$= \frac{5.0 - [4.0 \times 0.0072]}{0.1}$$

$$= \frac{5.0 - 0.03}{0.1}$$

$$= 49.7$$

VIII Dilution Water Check

A five-day BOD on unseeded dilution water must not be greater than 0.2 mg/L (and preferably not more than 0.1 mg/L). If it is greater than 0.2 mg/L check for contamination in the distilled water and, or, dirty BOD bottles. Do not use the value as a correction on the BOD.

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1 Standard Methods for the Examination of Wastewater, 14th ed, APHA, AWWA, WPCF, New York, pg 543, 1975.

This outline was prepared by Charles R. Feldmann, Chemist, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Analytical Techniques, Biochemical Oxygen Demand, Chemical Analysis, Laboratory Tests, Water Analysis

DATA SHEET

Initial DO in mg/L DOi = ______________

Final DO in mg/L DOf = ______________

% sample dilution expressed
as a decimal (e.g., 18% = 0.18) = ______________

mg 5-day unseeded BOD in mg/L = $\frac{\text{DOi} - \text{DOf}}{\text{decimal}}$

= _______ - _______

= __________

OPERATING CHARACTERISTICS AND USE OF THE pH METER

I INTRODUCTION

pH is a term used to describe the intensity of the acid or alkaline condition of a solution. The concept of pH evolved from a series of developments that led to a fuller understanding of acids and alkaline solutions (bases). Acids and bases were originally distinguished by their difference in physical characteristics (acids-sour, bases-soapy feel). In the 18th century it was recognized that acids have a sour taste (vinegar-acetic acid), that they react with limestone with the liberation of a gaseous substance (carbon dioxide) and that neutral substances result from their interaction with alkaline solutions.

Acids are also described as compounds that yield hydrogen ions when dissolved in water. And that bases yield hydroxide ions when dissolved in water. The process of neutralization is then considered to be the union of hydrogen (H^+) ions and hydroxyl (OH^-) ions to form neutral water ($H^+ + OH^- \longrightarrow H_2O$).

It has been determined that there are 1/10,000,000 grams of hydrogen ions and 17/10,000,000 grams of hydroxyl ions in one liter of pure water. The product of the H^+ and OH^- ions equal a constant value. Therefore, if the concentration of the H^+ ions is increased there is a corresponding decrease in OH^- ions. The acidity or alkalinity, hydrogen ion concentration of a solution is given in terms of pH. The pH scale extends from 0 to 14 with the neutral point at 7.0.

II INSTRUMENTATION

A General

Because of the differences between the many makes and model of pH meters which are available commercially, it is impossible to provide detailed instructions for the correct operation of every instrument. In each case, follow the manufacturer's instructions. Thoroughly wet the glass electrode and the calomel electrode and prepare for use in accordance with the instructions given. Standardize the instrument against a buffer solution with a pH approaching that of the sample, and then check the linearity of electrode response against at least one additional buffer of a different pH. The readings with the additional buffers will afford a rough idea of the limits of accuracy to be expected of the instrument and the technic of operation.

B Electrode Design

About 1925 it was discovered that an electrode could be constructed of glass which would develop a potential related to the hydrogen-ion concentration without interference from most other ions. The glass pH electrode is the nearest approach to a universal pH indicator known at present. It works on the principle of establishing a potential across a pH-sensitive, glass membrane whose magnitude is proportional to the difference in pH of the solution separated by this membrane.

All glass pH indicating electrodes have a similar basic design. Contained on one side of an appropriate glass membrane is a solution of constant pH. In contact with the other side of this pH sensitive glass is the solution of unknown pH. Between the surfaces of the glass membrane, a potential is established which is proportional to the pH difference of these solutions. As the pH of one solution is constant, this developed potential is a measure of the pH of the other.

To measure this potential, a half-cell is introduced into both the constant, internal solution and into the unknown, external solution. These half-cells are in turn connected to your pH meter. The internal reversible half-cell sealed within the chamber of constant pH is almost exclusively a wire of silver-silver chloride. The external reversible half-cell is often silver-silver chloride. If both the internal and external electrodes are combined in a common pH measuring device, the electrode is a combination pH electrode.

As the function of these half-cells is to provide a steady reference voltage against which voltage changes at the glass pH sensitive membrane can be referred, they must be protected from contamination and dilution by the unknown solutions. This is accomplished by permanently sealing the internal half-cell in a separate chamber which makes electrical contact to the unknown solution through a porous ceramic plug. This ceramic plug allows current to flow, but does not permit exchange of solution to this chamber. Gradually the kcL solution is slowly lost, therefore a filling port is placed in this electrode so that additional saturated potassium chloride can be added.

C Instrument Calibration

The pH balance control, by adding a voltage in series with the pH electrode system, allows the operator to adjust the meter readout to conform to the pH of the calibrating buffer. In general, calibrate the meter in the general range of the unknown solution. Appropriate buffers can be selected (pH 4.0, 6.8, 7.4 and 10.0). Always set the temperature compensator on the instrument to the temperature of the standard buffer solution.

For most accurate analysis the pH of the sample should be determined, and then buffered solutions of a pH above and below the determined pH should be selected to re-calibrate the instrument and the determination of the pH of the sample repeated for a final reading.

D Precision and Accuracy

The precision and accuracy attainable with a given pH meter will depend upon the type and condition of the instrument employed and the technique of standardization and operation. With the proper care, a precision of ± 0.02 pH unit and an accuracy of ± 0.05 pH unit can be achieved with many of the new and improved models. However, ± 0.1 pH unit represents the limit of accuracy under normal conditions. For this reason, pH values generally should be reported to the nearest 0.1 pH unit.

E Maintenance Practices

The reference chamber of the pH electrode system should always be kept nearly full of saturated kcL solution. Routinely check the level and saturation of potassium chloride in this reference chamber and add saturated kcL if necessary.

The pH sensitive glass membrane dehydrates when removed from water, and thus it is imperative that dry electrodes be soaked in buffer or water for several hours before use. To avoid this break-in period always keep the glass pH sensitive membrane wet between periods of use.

The buffers are pH standards; do not contaminate them.

If the meter is a battery operated instrument. To conserve the battery life, the instrument should be turned off when not in use.

III PROCEDURE

In the measurement of pH values of industrial wastes, effluents, sludges and similar samples, the electrodes must be thoroughly rinsed with buffer solution between samples and after calibrating. Buffer solutions can be prepared by use of the formulations shown in Table I.

The electrodes should be kept free of oil and grease and stored in water when not in use. In testing samples containing gaseous or volatile components which affect the pH value, any handling technic such as stirring or heating may cause loss of such components and thereby introduce error. For example, the loss of carbon dioxide from an anaerobic sludge digester sample due to stirring will result in an observed pH value which is too

Table I

Preparation of pH Standard Solutions

Standard Solution (Molality)	pH at 25°C	Weight of Chemicals Needed per 1,000 ml of Aqueous Solution at 25°C
Primary standards		
Potassium hydrogen tartrate (saturated at 25°C)	3.557	6.4g$KHC_4H_4O_6$*
0.05 potassium dihydrogen citrate	3.776	11.41g$KH_2C_6H_5O_7$
0.05 potassium hydrogen phthalate	4.008	10.12g$KHC_8H_4O_4$
0.025 potassium dihydrogen phosphate + 0.025 disodium hydrogen phosphate	6.865	3.388gKH_2PO_4† + 3.533gNa_2HPO_4†‡
0.008695 potassium dihydrogen phosphate + 0.03043 disodium hydrogen phosphate	7.413	1.179gKH_2PO_4† + 4.302gNa_2HPO_4†‡
0.01 sodium borate decahydrate (borax)	9.180	3.80g$Na_2B_4O_7 \cdot 10H_2O$‡
0.025 sodium bicarbonate + 0.025 sodium carbonate	10.012	2.092g$NaHCO_3$ + 2.640gNa_2CO_3
Secondary Standards		
0.05 potassium tetroxalate dihydrate	1.679	12.61g$KH_3C_4O_8 \cdot 2H_2O$
Calcium hydroxide (saturated at 25°C)	12.454	1.5g$Ca(OH)_2$*

---

*Approximate solubility

†Dry chemical at 110-130°C for 2 hr.

‡Prepare with freshly boiled and cooled distilled water (carbon dioxide-free)

high. If a sample of sludge or mud is highly buffered, a small amount of water may be added but the result cannot be considered valid unless further dilutions yield the same pH value. All dilutions should be reported along with the result.

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2 Sawyer, C. N., and McCarty, P. L. Chem. for San. Eng. 2nd Ed. McGraw-Hill, New York 1967

3 Instruction - Manual IL 175 Porto-matic pH meter, Instrumentation Laboratory, Inc. Lexington, Massachusetts. Standard Methods, APHA-AWWA-WPCF.. 14th Ed., 1975.

This outline was prepared by P. F. Hallbach, Chemist, National Training Center, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Hydrogen Ion Concentration, Instrumentation, Chemical Analysis

TURBIDITY

I INTRODUCTION

Turbidity as a water quality index refers to the degree of cloudiness present. Conversely, it is an index of clarity.

A Definition(1)

Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through samples of water.

B Relationship to Suspended Solids

This optical property, turbidity, is caused by suspended matter. The size, shape and reflection/absorption properties of that matter (not its weight) determine the degree of optical effects. It is very possible to have water with high turbidity but very low mg/L suspended solids. Thus one cannot use turbidity results to estimate the weight concentration and specific gravity of the suspended matter.

C Causes

1 clay, sand

2 silt, erosion products

3 microscopic and macroscopic organisms

4 finely divided organic products

5 others

D Effects on Water Quality(2)

Turbidity is an indicator of possible suspended matter effects such as impeding effective chlorine disinfection and clogging fish gills. However, the following list is limited to those effects associated with the optical (clarity) nature of turbidity.

1 Reducing clarity in water

- a drinking water quality
- b food processing
- c industrial processes
- d fish (seeing natural food)
- e swimming/water sports

2 Obscuring objects in water

- a submerged hazards
- b water sports

3 Light penetration

- a affects depth of compensation point for photosynthetic activity (primary food production).

4 Thermal Effects

High turbidity causes near surface waters to become heated because of the heat absorbancy of the particulate matter.

a Results in lower rate of oxygen transfer from air to water.

b Stabilizes water column and prevents vertical mixing.

  1 decreases downward dispersion of dissolved oxygen

  2 decreases downward dispersion of nutrients

E Criteria for Standards(2)

1 Finished Drinking Water - Maximum of one unit where the water enters the distribution system. The proposed(3) standard is one unit monthly average and five units average of two consecutive days. Under certain conditions a five unit monthly average may apply at state option.

2 For Freshwater Aquatic Life and Wildlife - The combined effect of color and turbidity should not change the

compensation point more than 10% from its seasonally established norm, nor should such a change place more than 10% of the biomass of photosynthetic organisms below the compensation point.

3 Turbidity Criteria Used by Industries:

a Textiles - 0.3 to 5 units

b Paper and allied products - Ranges from 10 to 100 units, depending on type of paper.

c Canned, dried and frozen fruits and vegetables - Same as for finished drinking water (1 turbidity unit).

F Processes to Remove Turbidity (Solids)

1 Coagulation

a pre-chlorination enhances coagulation

2 Sedimentation

3 Filtration

4 Aeration

5 Others

II VISUAL METHODS TO ESTIMATE TURBIDITY

A Early Efforts

In the early 1900's, Whipple and Jackson measured turbidity and developed a calibration scale for turbidity instruments.

B Jackson Candle Turbidimeter

Later Jackson developed apparatus which utilized the same "extinction" principle as the instrument devised earlier with Whipple.

1 Instrument

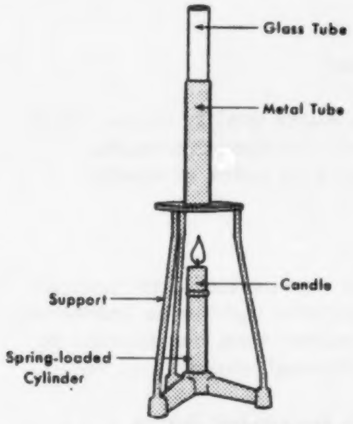

Figure 1 JACKSON CANDLE TURBIDIMETER

The sample was poured into a flat-bottomed, graduated glass tube held over a special candle. A turbidity reading was taken when the operator, observing from the top of the tube, saw the image of the candle flame disappear into a uniform glow. The reading related the final depth of sample in the tube with tube calibrations obtained from a standard suspension solution.

2 Standard Suspension

The standard was a suspension of silica prepared from Fuller's or diatomaceous earth. This was diluted to prepare a series of standard suspensions to graduate the turbidimeter. Graduations on all Jackson turbidimeters are made in conformity to this original data. Other suspensions are standardized by using the pre-calibrated turbidimeter and diluting accordingly.

3 Unit Used

Jackson Turbidity Unit (JTU) - parts per million suspended silica turbidity.

4 Standardization of Apparatus

The current edition of Standard Methods(1) contains specifications for the three essential components, i.e., the calibrated glass tube, the candle and a support.

5 Current Standard Suspension Solutions(1)

1 Natural turbid water from the same source as that tested gives best results. Determine turbidity with the instrument, then dilute to values desired.

2 The supernatant of a settled solution of kaolin is also used as a standard.

6 Limitations of Method

a Apparatus - difficult to exactly reproduce flame as to intensity and actual light path length. In general, it is a rather crude instrument with several variables that affect accuracy.

b Very fine suspended particles do not tend to scatter light of the longer wavelengths produced by the candle.

c Very dark and black particles can absorb enough light in comparison to the scattering of light to cause an incorrect reading of image extinction.

d Turbidities below 25 JTU cannot be directly measured. For lower turbidities (as in treated waters), indirect secondary methods are required to estimate turbidities.

C Hellige Turbidimeter(4)

This instrument utilizes the same extinction principle as the Jackson Candle Turbidimeter.

1 Equipment

An opal glass bulb supplies the light which is reflected (usually through a filter) upward through the sample which is contained in a glass tube. The entire system is enclosed in a black metal box. The operator views the sample by looking downward through an ocular tube screwed into the top of the box and adjusts the brightness of a central field of light by turning a calibrated dial on the outside of the apparatus. The point of uniform light intensity occurs when a black spot in the center of the field just disappears.

2 Range of Applicability

The equipment offers a choice of bulbs, filters and volumes of sample tubes. The variety affords a means to directly measure turbidity ranging from 0 through 150. The ranges can be extended by dilution.

3 Results

The final reading from the dial is translated into ppm silica turbidity units by using a graph corresponding to the bulb, filter and volume of sample used.

4 Standard Suspension Solution

Standardizing suspensions are not used by the operator. The graphs are supplied by the company for each instrument.

D Secchi Disk(5)

This is a very simple device used in the field to estimate the depth of visibility (clarity) in water.

1 Equipment

The disk is a weighted circular plate, 20 cm in diameter, with opposing black and white quarters painted on the surface. The plate is attached to a calibrated line by means of a ring on its center to assure that it hangs horizontally.

2 Readings

The disk is lowered into water until it disappears, lowered farther then raised until it reappears. The corresponding visibility depth (s) are determined from the calibrated line. Some read both depths and average them. Some read only the reappearance depth.

3 Standardizing the Procedure

There are many variables (position of sun and of observer, roughness of body of water, etc.) that affect readings. However, the same observer using a standard set of operating conditions can provide useful data to compare the visibility of different bodies of water.

4 Application of Results

Limnologists have found it convenient to establish a Secchi disk "factor" for estimating the photic depth where light intensity is about 1 per cent of full sunlight intensity. The true photic depth is determined by use of a submarine photometer and at the same time the observer takes a series of Secchi disk readings to obtain an average. Dividing the true photic depth by this average gives a factor which can be used to multiply other disk readings for an approximation of photic depth.

E Status of Visual Methods for Compliance Monitoring

The Federal Register(6) "List of Approved Methods" does not include any of these visual methods for National Pollutant Discharge Elimination System (NPDES) requirements. The visual methods are not recognized in the Federal Register(3) issue on Interim Drinking Water Regulations, either.

III NEPHELOMETRIC MEASUREMENTS FOR COMPLIANCE MONITORING

The subjectivity and apparatus deficiencies involved in visual methods of measuring turbidity make each unsuitable as a standard method.

Since turbidity is an expression of the optical property of scattering or absorbing light, it was natural that optical instruments with photometers would be developed for this measurement.

The type of equipment specified for compliance monitoring(3, 6) utilizes nephelometry.

A Basic Principle(7)

The intensity of light scattered by the sample is compared (under defined conditions) with the intensity of light scattered by a standard reference solution (formazin). The greater the intensity of scattered light, the greater the turbidity. Readings are made and reported in NTUs (Nephelometric Turbidity Units).

B Schematic

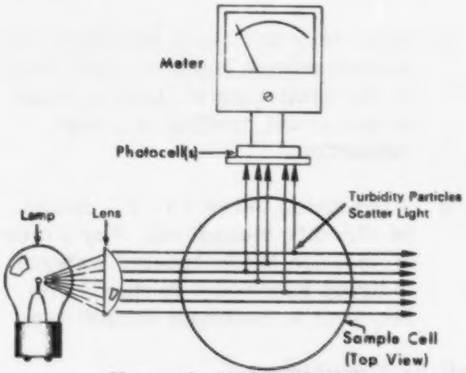

Figure 2 NEPHELOMETER (90° Scatter)

Light passes through a polarizing lens and on to the sample in a cell. Suspended particles (turbidity) in the sample scatter the light.

Photocell (s) detect light scattered by the particles at a 90° angle to the path of the incident light. This light energy is converted to an electric signal for the meter to measure.

1 Direction of Entry of Incident Light to Cell

a The lamp might be positioned as shown in the schematic so the beam enters a sample horizontally.

b Another instrument design has the light beam entering the sample (in a flat-bottom cell) in a vertical direction with the photocell positioned accordingly at a 90° angle to the path of incident light.

2 Number of Photocells

The schematic shows the photocell (s) at one 90 degree angle to the path of the incident light. An instrument might utilize more than one photocell position, with each final position being at a 90 degree angle to the sample liquid.

3 Meter Systems

a The meter might measure the signal from the scattered light intensity only.

b The meter might measure the signal from a ratio of the scattered light versus light transmitted directly through the sample to a photocell.

4 Meter Scales and Calbration

a The meter may already be calibrated in NTUs. In this case, at least one standard is run in each instrument range to be used in order to check the accuracy of the calibration scales.

b If a pre-calibrated scale is not supplied, a calibration curve is prepared for each range of the instrument by using appropriate dilutions of the standard turbidity suspension.

C EPA Specifications for Instrument Design(7)

Even when the same suspension is used for calibration of different nephelometers, differences in physical design of the turbidimeters will cause differences in measured values for the turbidity of the same sample. To minimize such differences, the following design criteria have been specified by the U. S. Environmental Protection Agency.

1 Defined Specifications

a Light Source

Tungsten lamp operated at a color temperature between 2200-3000°K.

b Distance Traveled by Light

The total of the distance traversed by the incident light plus scattered light within the sample tube should not exceed 10 cm.

c Angle of Light Acceptance of the Detector

Detector centered at 90° to the incident light path and not to exceed ± 30° from 90°.

The detector, and filter system if used, shall have a spectral peak response between 400 and 600 nm.

d Applicable Range

The maximum turbidity to be measured is 40 units. Several ranges will be necessary to obtain adequate coverage. Use dilution for samples if their turbidity exceeds 40 units.

2 Other EPA Design Specifications

a Stray Light

Minimal stray light should reach the detector in the absence of turbidity.

Some causes of stray light reaching the photocell (s) are:

1 Scratches or imperfections in glass cell windows.

2 Dirt, film or condensation on the glass.

3 Light leakages in the instrument system.

A schematic of these causes is shown in Figure 3.

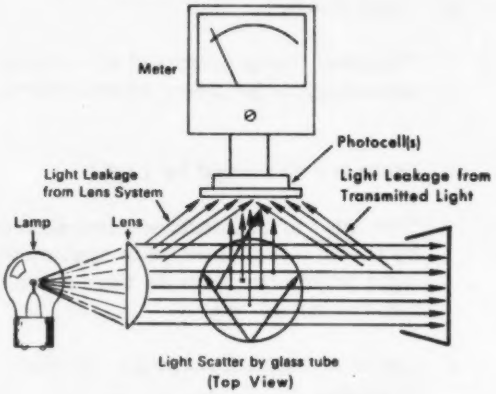
Meter
Photocell(s)
Light Leakage from Lens System
Light Leakage from Transmitted Light
Lamp
Lens
Light Scatter by glass tube (Top View)

Figure 3 NEPHELOMETER SOURCES OF STRAY LIGHT

Stray light error can be as much as 0.5 NTU. Remedies are close inspection of sample cells for imperfections and dirt, and good design which can minimize the effect of stray light by controlling the angle at which it reaches the sample.

b Drift

The turbidimeter should be free from significant drift after a short warm-up period. This is imperative if the analyst is relying on a manufacturer's solid scattering standard for setting overall instrument sensitivity for all ranges.

c Sensitivity

In waters having turbidities less than one unit, the instrument should detect turbidity differences of 0.02 unit or less. Several ranges will be necessary to obtain sufficient sensitivity for low turbidities.

3 Examples of instruments meeting the specifications listed in 1 and 2 above include:

a Hach Turbidimeter Model 2100 and 2100 A

b Hydroflow Instruments DRT 100, 200, and 1000

4 Other turbidimeters(12) meeting the listed specifications are also acceptable.

D Sources of Error

1 Marred Sample Cells

a Discard scratched or etched cells.

b Do not touch cells where light strikes them in instrument.

c Keep cells scrupulously clean, inside and out.(8)

1 Use detergent solution.

2 Organic solvents may also be used.

3 Use deionized water rinses.

4 Rinse and dry with alcohol or acetone.

2 Standardizing Suspensions(7)

a Use turbidity - free water for preparations. Filter distilled water through a 0.45 μ m pore size membrane filter if such filtered water shows a lower turbidity than the distilled water.

b Prepare a new stock suspension of Formazin each month.

c Prepare a new standard suspension and dilutions of Formazin each week.

3 Sample Interferences

a Positive

1 Finely divided air bubbles

b Negative

1 Floating debris

2 Coarse sediments (settle)

3 Colored dissolved substances (absorb light)

E Reporting Results(7)

NTU	Record to Nearest:
0.0-1.0	0.05
1-10	0.1
10-40	1
40-100	5
100-400	10
400-1000	50
>1000	100

F Precision and Accuracy(7)

1 In a single laboratory (MDQARL), using surface water samples at levels of 26, 41, 75 and 180 NTU, the standard deviations were ± 0.60, ± 0.94, ± 1.2 and ± 4.7 units, respectively.

2 Accuracy data is not available at this time.

IV STANDARD SUSPENSIONS AND RELATED UNITS(9)

One of the critical problems in measuring turbidity has been to find a material which can be made into a reproducible suspension with uniform sized particles. Various materials have been used.

A Natural Materials

1 Diatomaceous earth

2 Fuller's earth

3 Kaolin

4 Naturally turbid waters

Such suspensions are not suitable as reproducible standards because there is no way to control the size of the suspended particles.

B Other Materials

1 Ground glass

2 Microorganisms

3 Barium sulfate

4 Latex spheres

Suspensions of these also proved inadequate.

C Formazin

1 A polymer formed by reacting hydrazine sulfate and hexamethylenetetramine sulfate.

2 It is more reproducible than previously-used standards. Accuracy of ± one per cent for replicate solutions has been reported.

3 In 1958, the Association of Analytical Chemists initiated a standardized system of turbidity measurements for the brewing industry by:

a defining a standard formula for making stock Formazin solutions and

b designating a unit of measurement based on Formazin, i.e., the Formazin Turbidity Unit (FTU).

4 During the 1960's Formazin was increasingly used for water quality turbidity testing. It is the currently recognized standard for compliance turbidity measurements.

D Units

1 At first results were translated into Jackson Turbidity Units (JTU). However, the JTU was derived from a visual measurement using concentrations (mg/liter) of silica suspensions prepared by Jackson. They have no direct relationship to the intensity of light scattered at 90 degrees in a nephelometer.

2 For a few years, results of nephelometric measurements using specified Formazin standards were reported directly as Turbidity Units (TU's).

3 Currently, the unit used is named according to the instrument used for measuring turbidity. Specified Formazin standards are used to calibrate the instrument and results are reported as Nephelometric Turbidity Units (NTUs).

V TURBIDITY MEASUREMENTS FOR PROCESS CONTROL

The schematic and design characteristics discussed above for nephelometric instruments is the required method for measuring turbidity for compliance purposes. Turbidity data is also widely used to check water for process design purposes and to monitor water for process control purposes. The nature of the liquids to be monitored, and the degree of sensitivity required for signalling the remedy to be applied have led to the development of monitoring instrumentation that differs in design or in principle from the instrument previously described.

A Users of Control Data

1 Potable Water Treatment Plants

2 Municipal Wastewater Treatment Plants

3 Industrial Processers

B Applications of Control Data (10, 11)

1 Coagulation Processes

a To check the effectiveness of different coagulants.

b To check the effectiveness of different dosages.

c To regulate chemical dosages by automating chemical feed controls.

2 Settling Processes

a To determine intermittent need for settling processes.

b To control the sludge blanket height in activated sludge treatment processes.

c To activate removal and re-cycling of very high density sludge from settling tanks.

d To monitor effectiveness of settling processes.

3 Filtration Processes

a To determine intermittent need for filtration.

b To facilitate high rate filtration processes.

c To prevent excessive loadings for filtration systems.

d To check the efficiency of filtration systems.

e To regulate filter backwash operations.

4 Rust in Water Distribution Systems

a To locate sources of contamination.

b To monitor intermittent occurrences.

5 Steam Boiler Operations

a To detect corrosion products in boiler water.

b To detect evidences of corrosion in condensates.

c To determine the effectiveness of corrosion treatment measures.

C Varieties of Instrumentation

1 Surface Scatter Nephelometers

In forward - scattering instruments, the angle of the incident light is adjusted to illuminate the surface of a smooth flowing liquid at an angle of about 15 degrees from horizontal, rather than beamed through a glass cell of the liquid as described for a nephelometer earlier in this outline. A photocell is located immediately above the illuminated area so that vertically scattered light from turbidity in the sample reaches it.

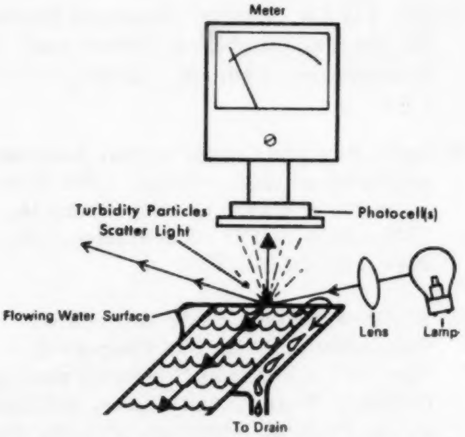

Figure 4 NEPHELOMETER (Surface Scatter)

Variations of the methodology include sidescatter and backscatter designs.

a Advantages

1 No glass sample cells are used. Attendant problems of cleanliness and condensation are eliminated. The surface of the liquid provides a near-perfect optical surface which is difficult to achieve in glass cells.

2 Stray light effects on the photocell are minimized because the simpler design eliminates some of the sources of stray light.

3 Since flowing sample is used, interferences from air bubbles and/or floating materials are quickly eliminated.

4 This design is sensitive to the presence of larger suspended particles.

b Disadvantage

As turbidity becomes high, penetration of incident light decreases to cause a falling off of response.

2 Absorption Spectrophotometry

The incident light is beamed through a smooth, flat stream of sample and the transmitted light (in contrast to nephelometric scattered light) is measured by a spectrophotometer. A schematic is shown in Figure 5.

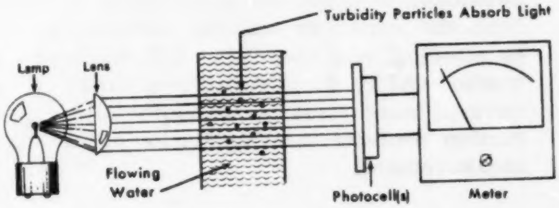

Figure 5 ABSORPTION SPECTROPHOTOMETRY

a Advantages

1 No glass sample cells are used.

2 The simpler design eliminates sources of stray light.

3 Applicable to measure high turbidities, e.g., in sludges.

b Disadvantage

1 Low sensitivity for many applications.

2 Color constituents interfere.

VI SUMMARY

Turbidity measurements represent the optical property of light scattering by suspended solids. NTUs are an index of the effects of the size, etc., of suspended particles but cannot be used to indicate mg/L quantities of those particles. The parameter is required for finished potable water and is extremely useful in reference to aesthetic quality (clarity), photic conditions and thermal effects in bodies of water. It is also widely applied for process control of water and wastewater treatment and of industrial processes.

There have been difficulties in developing a satisfactory standard method for this measurement. Early methods depended on a subjective judgement of an extinction point where transmitted light balanced scattered light in rather crude apparatus. Although the apparatus was refined and standardized to a large extent, the subjectivity of these visual methods was still an unsatisfactory element of such methodology.

Eventually, optical instrumentation was developed to eliminate subjectivity from the measurement. Nephelometry (scattering) was chosen for the standard method and U. S. EPA has specified several instrument design criteria to further promote standardization of the measurement.

Finding a suitable (reproducible) standard suspension has also been a problem. Currently, Formazin is specified as the standard because, to date, it is more reproducible than other suspensions proved to be.

Establishing a meaningful unit progressed along with development of instrumentation and agreement on a standard suspension. The current unit (NTU) is derived from the method of measurement, nephelometry, and use of a standard Formazin suspension.

Even with the efforts to standardize instrument design, to find a suitable standard suspension, and to agree on a meaningful unit, there are still problems about this measurement. Instruments meeting the design criteria and standardized with Formazin suspensions can give turbidity readings differing significantly for the same sample.

Another problem area is associated with sample dilutions. Work has indicated a progressive error on sample turbidities in excess of 40 units, so such samples are to be diluted. However, obtaining a dilution exactly representative of the original suspension is difficult to achieve. Thus dilutions often significantly fail to give linearly decreased results when re-measured.

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This outline was prepared by Audrey D. Kroner, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Chemical Analysis, Instrumentation, Secchi disks, Turbidity, Wastewater, Water Analysis

CALIBRATION AND USE OF A TURBIDIMETER (NEPHELOMETER)

I REAGENTS[1]

A Turbidity - free water - Pass distilled water through a 0.45 μm pore size membrane filter if such filtered water shows a lower turbidity than the original distilled water.

B Stock Turbidity Suspension - 400 units

1 Directions are for preparing 100.0 ml. Larger volumes may be required.

2 Solution, 1: Dissolve 1.00g hydrazine sulfate, $(NH_2)_2 \cdot H_2SO_4$, in turbidity free water and dilute to 100ml in a volumetric flask.

3 Solution 2: Dissolve 10.00g hexamethylenetetramine in turbidity-free water and dilute to 100ml in a volumetric flask.

4 Suspension: Mix 5.0ml Solution 1 with 5.0ml Solution 2 in a 100 ml volumetric flask. Allow to stand for 24 hours at 25 ± 3°C. Dilute to the mark and mix.

5 Stability: Prepare a new stock suspension each month.

C Standard Turbidity Suspension - 40 units

1 Dilute 10.00ml stock turbidity suspension to 100ml with turbidity - free water in a 100 ml volumetric flask. The turbidity is defined as 40 units.

2 Stability: Prepare a new standard suspension each week.

D Dilute Standard Turbidity Suspension - 4 units

1 Dilute 1.0 ml stock turbidity suspension to 100 ml with turbidity-free water in a 100 ml volumetric flask. The turbidity should be 4 units.

2 Stability: Prepare a new standard suspension each week.

E Secondary Standards

1 Solutions standardized with Formazin can be purchased from the manufacturer of the instrument.

2 Date such solutions. Store under the conditions specified. Discard and replace when flocculation in the solution is observed or when it fails a periodic check with a Formazin Standard. (2)(3)

II PREPARATIONS FOR MEASUREMENTS

A Suspensions

1 Check date of preparation and prepare fresh solutions if required.

B Sample Cell (5)

1 Cells should be cleaned immediately after use as described in V.B. below.

2 Inspect cells for cleanliness. If necessary, clean them using V.B. below.

3 Check cells for scratches and etching. Discard those with imperfections.

C Instrument

1 Scale - If a scale is inserted, check that it is in the correct position. If the scale is blank, construct a calibration scale for each range on the instrument. (See III B).

2 Zero - Adjust meter needle to zero point on scale as directed by manufactuer.

3 Lens - Check for cleanness. If required, follow manufacturer's instructions for removing and cleaning the lens. Accurate re-positioning of the lens is critical for accurate measurements.

4 Warm-Up Period - Follow manufacturer's instructions. Continuous running is often suggested because of the photomultiplier tubes.

5 Focus - Use template or method described by manufacturer to check and, if necessary, set the focus.

D Determine Range of Sample Turbidity

1 Use steps 5 through 16 in III A below EXCEPT Step 12 which should be: Obtain a turbidity reading from the scale.

2 Note which Range (instrument scale) best "brackets" the turbidity of each sample.

3 If the turbidity of a sample exceeds 40 units, use the higher scales provided to determine the dilution required so the final reading will be below 40 units. For final measurements, use the Range (Scale) appropriate for the diluted sample.

III INSTRUMENT CALIBRATION AND MEASUREMENTS[(1)]

A Pre-Calibrated Scale

1 Each week, prepare at least one standard for the required instrument range (s) (as determined in II. D. above) by diluting one of the Formazin suspensions described above in I. Reagents. The table below gives "EXAMPLE DILUTIONS".

2 Set the instrument RANGE knob at the first range to be tested. (The instrument should be ON, warmed up, zeroed, etc., as in II C above).

3 Make any instrument adjustment specified by the manufacturer to use this RANGE.

4 Rinse the SAMPLE CELL 2 times with the appropriate suspension (or sample).

5 Shake the suspension to thoroughly disperse the solids. (For secondary standards, check the manufacturer's instructions for this step).

EXAMPLE DILUTIONS

NO.	EXAMPLE INSTRUMENT RANGES	VOLUME	TURBIDITY STANDARD	FINAL DILUTION	FINAL TURBIDITY
1	0 - 0.1	2.5 ml	4 unit	100.0 ml	0.1
2	0 - 0.2	5.0 ml	4 unit	100.0 ml	0.2
3	0 - 0.3	7.5 ml	4 unit	100.0 ml	0.3
4	0 - 1	2.5 ml	40 unit	100.0 ml	1
5	0 - 3	7.5 ml	40 unit	100.0 ml	3
6	0 - 10	25.0 ml	40 unit	100.0 ml	10
7	0 - 30	7.5 ml	400 unit	100.0 ml	30
8	0 - 100	25 ml	400 unit	100.0 ml	100
9	0 - 300	75 ml	400 unit	100.0 ml	300
10	0 - 400	100 ml	400 unit	100.0 ml	400
11	0 - 1000	100 ml	400 unit	100.0 ml	400

6 Wait until air bubbles disappear in the suspension.

7 Pour the suspension into the SAMPLE CELL up to the level specified by the manufacturer. CAUTION: Always hold the cell above the area from which light scattering is measured.

8 If applicable, screw cap on cell.

9 Wipe the outside of the cell with a lint-free tissue.

10 Examine the suspension in the cell to check for air bubbles. If air bubbles are present, eliminate them:

   a by inserting the cell (5) in the sample holder and waiting a few minutes so bubbles rise above photo-multiplier tube. CAUTION: More bubbles can form if a temperature rise occurs.

   b by holding the cell at the top and:

      1 flicking side with your finger or

      2 dipping the end of the cell into an ultrasonic cleaning bath or

      3 centrifuging the filled cell in cups with rubber cushions and surrounded with water.

      4 NOTE: After any of these remedies, again wipe the outside of the cell. When air bubbles are gone, insert the (5) cell in the sample holder.

11 Place the LIGHT SHIELD according to the manufacturer's instruction.

12 Use the STANDARDIZING control to obtain a meter reading corresponding to the turbidity of the standard suspension.

13 Remove the LIGHT SHIELD.

14 Remove the SAMPLE CELL.

15 Discard the standard suspension.

16 Rinse SAMPLE CELL 2 times with turbidity - free water.

17 Use Steps 4 through 11 for each sample (or diluted sample) to be tested in this range. For samples, step 12 should be : Record the turbidity reading for the sample. Then do Steps 13 through 16 as above.

NOTE: The final reading for samples should not exceed 40 NTU. If this reading is exceeded for a sample, dilute it and repeat the calibration/ measurement procedure above using the appropriate range and standard. (Selection of the range as described in II. D. above should make this unnecessary at this stage of the procedure).

B Non-Calibrated Scale

Prepare a series of standards and make a calibration scale for each range of the instrument.

   a The instrument should be ON, warmed up, zeroed, etc., as in II C above.

   b Prepare enough standards to give several points on each scale so estimated readings can be reasonably accurate.

   c Use the table of EXAMPLE DILUTIONS in III A above to prepare the highest standard for each instrument range. The rest of each calibrating series can also be prepared by dilutions based on the information in the table.

2 Self-prepared scales should also be calibrated each day using the procedure given in III A above for pre-calibrated scales.

3 Self-prepared scales are used for samples in the manner described in III A above for pre-calibrated scales.

IV CALCULATION/RESULTS(1)

A Diluted Samples

Multiply final sample readings by the appropriate dilution factor.

B Reporting Results

Report results as follows:

NTU	Report to Nearest:
0.0 - 1.0	0.05
1 - 10	0.1
10 - 40	1
40 - 100	5
100 - 400	10
400 - 1000	50
> 1000	100

C Precision and Accuracy

1 In a single laboratory (MDQARL), using surface water samples at levels of 26, 41, 75 and 180 NTU, the standard deviations were ± 0.60, ± 0.94, ± 1.2 and ± 4.7 units, respectively.

2 Accuracy data is not available at this time.

V STORAGE

A Standard Suspensions

1 Store in glass containers at room temperature.

2 Excess light or heat may affect stability.

3 Observe stability times noted for each in I. above.

B Sample Cells

1 Discard cells with scratches or etching.

2 Clean cells immediately after use with this order of treatments:(4)

a detergent

b organic solvents, if required

c deionized water

d alcohol or acetone rinses to dry

e lint-free tissue, if required

3 Store in a manner to protect the cells from scratches.

C Instrument

1 A line operated instrument should be permanently located so moving it often is not necessary.

2 Turbidimeters should be protected from dust, especially the lens system.

3 Store any removable parts as directed by manufacturer.

4 Close any access doors.

5 Because of the photomultiplier tubes, the manufacturer may suggest continuous running of the instrument to insure maximum accuracy for measurements. Frequency of use can determine the actual routine for warm-up time.

6 Follow any other storage directions in the manufacturer's manual.

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5 The orientation of the sample cell should be identical each time it is placed in the instrument. Mark the cell and instrument to ensure that the cell is oriented consistently. Use the same cell throughout the procedure.

This outline was prepared by Audrey D. Kroner, Chemist, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Analytical Techniques, Laboratory Tests, Turbidity

CHEMICAL OXYGEN DEMAND AND COD/BOD RELATIONSHIPS

I DEFINITION

A The Chemical Oxygen Demand (COD) test is a measure of the oxygen equivalent of that portion of the organic matter in a sample that is susceptible to oxidation under specific conditions of oxidizing agent, temperature and time.

B A variety of terms have been and are used for the test described here as COD:

1. Oxygen absorbed (OA) primarily in British practice.
2. Oxygen consumed (OC) preferred by some, but unpopular.
3. Chemical oxygen demand (COD) current preference.
4. Complete oxygen demand (COD) misnomer.
5. Dichromate oxygen demand (DOC) earlier distinction of the current preference for COD by dichromate or a specified analysis such as Standard Methods.
6. Others have been and are being used. Since 1960, terms have been generally agreed upon within most professional groups as indicated in I-A and B-3 and the explanation in B-5.

C The concept of the COD is almost as old as the BOD. Many oxidants and variations in procedure have been proposed, but none have been completely satisfactory.

1. Ceric sulfate has been investigated, but in general it is not a strong oxidant.
2. Potassium permanganate was one of the earliest oxidants proposed and until recently appeared in Standard Methods (9th ed.) as a standard procedure. It is currently used in British practice as a 4-hr. test at room temperature.

   a The results obtained with permanganate were dependent upon concentration of reagent, time of oxidation, temperature, etc., so that results were not reproducible.

3. Potassium iodate or iodic acid is an excellent oxidant but methods employing this reaction are time-consuming and require a very close control.
4. A number of investigators have used potassium dichromate under a variety of conditions. The method proposed by Moore at SEC is the basis of the standard procedure.(1, 2) Statistical comparisons with other methods are described. (3)
5. Effective determination of elemental carbon in wastewater was sought by Buswell as a water quality criteria.

   a Van Slyke(4) described a carbon determination based on anhydrous samples and mixed oxidizing agents including sulfuric, chromic, iodic and phosphoric acids to obtain a yield comparable to the theoretical on a wide spectrum of components.

   b Van Hall, et al.,(5) used a heated combustion tube with infrared detection to determine carbon quickly and effectively by wet sample injection.

6. Current development shows a trend to instrumental methods automating

conventional procedures or to seek elemental or more specific group determination.

II RELATIONSHIP OF THE COD TEST WITH OTHER OXIDATION CRITERIA IS INDICATED IN TABLE 1.

A Table 1

Test	Test Temp. °C	Reaction time	Oxidation system	Variables
BOD	20	days	Biol. prod. Enz. Oxidn.	Compound, environment, biota, time, numbers. Metabolic acceptability, etc.
COD	145	2 hrs.	50% H_2SO_4 $K_2Cr_2O_7$ May be catalyzed	Susceptibility of the test sample to the specified oxidation
IDOD	20	15'	Diss. oxyg.	Includes materials rapidly oxidized by direct action, Fe^{+2}, SH.
Van Slyke Carbon detn.	400+	1 hr.	H_3PO_4 HIO_3 H_2SO_4 $K_2Cr_2O_7$ Anhydrous	Excellent approach to theoretical oxidation for most compounds (N-nil)
Carbon by combustion +IR	950	minutes	Oxygen atm. catalyzed	Comparable to theoretical for carbon only.
Chlorine Demand	20	20 min.	HOCl soln.	Good NH_3 oxidn. Variable for other compounds.

B From Table 1 it is apparent that oxidation is the only common item of this series of separate tests.

1 Any relationships among COD & BOD or any other tests are fortuitous because the tests measure the the oxidizability of a given sample under specified conditions, which are different for each test.

2 If the sample is primarily composed of compounds that are oxidized by both procedures (BOD and COD) a relationship may be established.

a The COD procedure may be substituted (with proper qualifications) for BOD or the COD may be used as an indication of the dilution required for setting up BOD analysis.

b If the sample is characterized by a predominance of material that can be chemically, but not biochemically oxidized, the COD will be greater than the BOD. Textile wastes, paper mill wastes, and other wastes containing high concentrations of cellulose have a high COD, low BOD.

c If the situation in item b is reversed the BOD will be higher than the COD. Distillery wastes or refinery wastes may have a high BOD, low COD, unless catalyzed by silver sulfate.

d Any relationship established as in 2a will change in response to sample history and environment. The BOD tends to decrease more rapidly than the COD. Biological cell mass or detritus produced by biological action has a low BOD but a relatively high COD. The COD/BOD ratio tends to increase with time, treatment, or conditions favoring stabilization.

III ADVANTAGES AND LIMITATIONS OF THE COD TEST(2) AS RELATED TO BOD

A Advantages

1 Time, manipulation, and equipment costs are lower for the COD test.

2 COD oxidation conditions are effective for a wider spectrum of chemical compounds.

3 COD test conditions can be standardized more readily to give more precise results.

4 COD results are available while the waste is in the plant, not several days later, hence, plant control is facilitated.

5 COD results are useful to indicate downstream damage potential in the form of sludge deposition.

6 The COD result plus the oxygen equivalent for ammonia and organic nitrogen is a good estimate of the ultimate BOD for many municipal wastewaters.

B Limitations

1 Results are not applicable for estimating BOD except as a result of experimental evidence by both methods on a given sample type.

2 Certain compounds are not susceptible to oxidation under COD conditions or are too volatile to remain in the oxidation flask long enough to be oxidized.

Ammonia, aromatic hydrocarbons, saturated hydrocarbons, pyridine, and toluene are examples of materials with a low analytical response in the COD test.

3 Dichromate in hot 50% sulfuric acid requires close control to maintain safety during manipulation.

4 Oxidation of chloride to chlorine is not closely related to BOD but may affect COD results.

5 It is not advisable to expect precise COD results on saline water.

IV BACKGROUND OF THE STANDARD METHODS COD PROCEDURE

A The COD procedure(1) considered dichromate oxidation in 33 and 50 percent sulfuric acid. Results indicated preference of the 50 percent acid concentration for oxidation of sample components. This is the basis for the present standard procedure.

B Muers(6) suggested addition of silver sulfate to catalyze oxidation of certain low molecular weight aliphatic acids and alcohols. The catalyst also improves oxidation of most other organic components to some extent but does not make the COD test universally applicable for all chemical pollutants.

C The unmodified COD test result (A) includes oxidation of chloride to chlorine. Each mg of chloride will have a COD equivalent of 0.23 mg. Chlorides must be determined in the sample and the COD result corrected accordingly.

1 For example, if a sample shows 300 mg of COD per liter and 200 mg Cl^- per liter the corrected COD result will be 300 -(200 x 0.23)or 300 - 46 = 254 mg COD/L on a chloride corrected basis

2 Silver sulfate addition as a catalyst tends to cause partial precipitation of silver chloride even in the hot acid solution. Chloride corrections are questionable unless the chloride is oxidized before addition of silver sulfate, i.e., reflux for 15 minutes for chloride oxidation, add Ag_2SO_4, and continue the reflux or use of $HgSO_4$(D).

D Dobbs and Williams (7) proposed prior complexation of chlorides with $HgSO_4$ to prevent chloride oxidation during the test. A ratio of about 10 of Hg^{++} to 1 of Cl^- (wt. basis) appears essential. The Cl^- must be complexed in acid solution before addition of dichromate and silver sulfate.

1 For unexplained reasons the $HgSO_4$ complexation does not completely prevent chloride oxidation in the presence of high chloride concentrations.

2 Factors have been developed to provide some estimate of error in the result due to incomplete control of chloride behavior. These tend to vary with the sample and technique employed.

E It is not likely that COD results will be precise for samples containing high chlorides. Sea water contains 18000 to 21000 mgCl^-/L normally. Equivalent chloride correction for COD exceeds 4000 mg/L The error in chloride determination may give negative COD results upon application of the correction.

Incomplete control of chloride oxidation with $HgSO_4$ may give equally confusing results.

$HgSO_4$ appears to give precise results for COD when chlorides do not exceed about 2000 mg/L Interference increases with increasing chlorides at higher levels.

F The 12th edition of Standard Methods reduced the amount of sample and reagents to 40% of amounts utilized in previous editions. There has been no change in the relative proportions in the test. This step was taken to reduce the cost of providing expensive mercury and silver sulfates required. Results are comparable as long as the proportions are identical. Smaller aliquots of sample and reagents require more care during manipulation to promote precision.

G The EPA Methods for COD

1 For routine level COD (samples having an organic carbon concentration greater than 50 mg/liter and a chloride concentration less than 2000 mg/liter), EPA has a procedure entitled, "Titrimetric, mid-level."

2 For low level COD (samples with less than 50 mg/liter organic carbon and chloride concentration less than 2000 mg/liter), EPA provides an analytical procedure (9). The difference from the routine procedure primarily involves a greater sample volume and more dilute solutions of dichromate and ferrous ammonium sulfate.

3 For saline samples (chloride level exceeds 1000 mg/liter), EPA provides an analytical procedure(9) involving preparation of a standard curve of COD versus mg/liter chloride to correct the calculations. Volumes and concentrations for the sample and reagents are adjusted for this type of determination

V PRECISION AND ACCURACY(9)

Eighty six analysts in 58 laboratories analyzed a distilled water solution containing oxidizable organic material equivalent to 270 mg/L COD. The standard deviation was ± 17.76 mg/L COD with an accuracy as percent relative error (bias) of –4.7%. For a solution equivalent to 12.3 mg/L COD (low level), the standard deviation was ± 4.15 mg/L with an accuracy as percent relative error (bias) of 0.3%. (EPA Method Research Study 3)

VI REMARKS PERTINENT TO EFFECTIVE COD DETERMINATIONS INCLUDE:

A Sample size and COD limits for 0.250 N reagents are approximately as given. For 0.025 N reagents multiply COD by 0.1. Use the weak reagent for COD's in the range of 5-50 mg/L. (low level).

Sample Size	mg COD/liter
20 ml	2000
10 ml	4000
5 ml	8000

B Most organic materials oxidize relatively rapidly under COD test conditions. A significant fraction of oxidation occurs during the heating upon addition of acid but the orange color of dichromate should remain. If the sample color changes from orange to green after acid addition the sample was too large. Discard without reflux and repeat with a smaller aliquot until the color after mixing does not go beyond a brownish hue. The dichromate color change is less rapid with sample components that are slowly oxidized under COD reaction conditions.

C Chloride concentrations should be known for all test samples so appropriate analytical techniques can be used.

D Special precautions advisable for the regular COD procdure and essential when using 0.025 N reagents include:

1 Keep the apparatus assembled when not in use.

2 Plug the condenser breather tube with glass wool to minimze dust entrance.

3 Wipe the upper part of the flask and lower part of the condenser with a wet towel before disassembly to minimize sample contamination.

4 Steam out the condenser after use for high concentration samples and periodically for regular samples. Use the regular blank reagent mix and heat, without use of condenser water, to clean the apparatus of residual oxidizable components.

5 Distilled water and sulfuric acid must be of very high quality to maintain low blanks on the refluxed samples for the 0.025 N oxidant.

VII NPDES METHODOLOGY

Under the National Pollutant Discharge Elimination System, the accepted method can be found in Standard Methods, ASTM and the EPA manual. See the current nd NPDES Guidelines for page numbers.

ACKNOWLEDGEMENT:

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This outline was prepared by F. J. Ludzack, Chemist, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Analysis, Biochemical Oxygen Demand, Chemical Analysis, Chemical Oxygen Demand, Chlorides, Oxygen Demand, Wastewater, Water Analysis

LABORATORY PROCEDURE FOR ROUTINE LEVEL CHEMICAL OXYGEN DEMAND

I REAGENTS

A Standard Potassium Dichromate (0.250 N):

Dissolve 12.259 g of primary standard grade $K_2Cr_2O_7$, which has been dried at 103° C for two hours, in distilled water and dilute to one liter.

B Ferrous Ammonium Sulfate (0.1N):

Dissolve 39 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water.
Carefully add 20 ml of concentrated H_2SO_4. Cool and dilute to one liter.

C Ferroin Indicator:

Dissolve 1.485 g 1,10-phenanthroline monohydrate and 0.695 g $FeSO_4 \cdot 7H_2O$ in water and dilute to 100 ml. This indicator may be purchased already prepared.

D Concentrated Sulfuric Acid (36 N):

E Mercuric Sulfate: Analytical Grade

F Silver Sulfate: Analytical Grade

G Concentrated Sulfuric Acid - Silver Sulfate:

Dissolve 22 g of silver sulfate in a 9 lb bottle of concentrated sulfuric acid. (4-5 hours, with stirring, for dissolution)

II EQUIPMENT PREPARATION

Before use, the Erlenmeyer flask (500 ml, 24/40 standard taper joint) and reflux condenser (300 mm jacket Liebig, West or equivalent) with 24/40 standard taper joint, should be steamed out to remove trace organic contaminants. Add 10 ml of 0.250 N $K_2Cr_2O_7$, 50 ml of distilled water, and several boiling stones to the flask. Carefully add 20 ml of concentrated H_2SO_4 and mix thoroughly. Connect the flask and condenser, but do not turn on the water to the condenser. Boil the mixture so that steam emerges from the top of the condenser for several minutes. Cool the mixture, carefully discard the acid, and rinse the condenser and flask with distilled water. In order to prevent contamination from air-borne particles, re-assemble the apparatus. The top of the condenser should be lightly plugged with glass wool during storage and use.

III STANDARDIZATION(1) OF FERROUS AMMONIUM SULFATE

A Measure 10.0 ml of the standard potassium dichromate sol. into a 500 ml, wide mouth Erlenmeyer flask.

B Add about 90 ml distilled water and mix.

C Add 30 ml of concentrated H_2SO_4 and cool the mixture to room temperature.

D Add 2-3 drops of ferroin indicator and titrate to a reddish-brown end point with the ferrous ammonium sulfate sol..

E Calculate the normality, N, of the ferrous ammonium sulfate sol.:

$$\text{N of } Fe(NH_4)_2(SO_4)_2 = \frac{\text{ml } K_2Cr_2O_7 \times \text{N of } K_2Cr_2O_7}{\text{ml } Fe(NH_4)_2(SO_4)_2}$$

IV PROCEDURE (1)

A Pipet 20 ml of sample (or pipet a smaller portion of sample and add enough distilled water to make a 20 ml volume) into a 500 ml 24/40 standard taper Erlenmeyer flask.

B Add 0.4 g mercuric sulfate.

C Add 6-10 boiling chips.

D Slowly add 3 ml of concentrated sulfuric acid and swirl to dissolve the mercuric sulfate. (Some solid may remain). Cool while mixing.

E Add 10.0 ml of the 0.250 N potassium dichromate sol. and mix.

F Attach the flask to the condenser and start the cooling water.

G Slowly add 27 ml of the sulfuric acid-silver sulfate reagent through the open end of the condenser. Try to mix the contents while adding the acid.

H CAUTION: After adding the acid, swirl the flask to thoroughly mix the contents. Otherwise, local heating can occur in the bottom of the flask and the mixture may be blown out of the condenser.

I Plug the open end of the condenser with glass wool or cover with a small beaker (depending on construction) to prevent intrusion of contaminants.

J Turn on the heat source and reflux the mixture for 2 hours after boiling begins.

K Turn off the heat and allow the solution to cool. Then turn off cooling water.

L Wash down the inside walls of the condenser with distilled water.

M With a twisting motion, disconnect the condenser from the flask, then rinse the joint so the rinses go into the flask.

N Add distilled water to the flask for a total volume of about 130 ml.

O If the mixture is still warm, cool to room temperature.

P Add 2-3 drops of ferroin indicator and mix.

Q Titrate the excess dichromate with standard ferrous ammonium sulfate sol. to a reddish brown end point.

R A blank consisting of 20 ml of distilled water and containing all the test reagents is to be refluxed and titrated in the same manner as the sample.

V CALCULATION

$$\text{mg COD/L} = \frac{(A-B)\ N \times 8 \times 1000}{\text{ml of sample}}$$

COD = chemical oxygen demand

A = ml Fe $(NH_4)_2(SO_4)_2$ used for blank

B = ml $Fe(NH_4)_2(SO_4)_2$ used for sample

N = N of $Fe(NH_4)_2(SO_4)_2$

8 = equivalent weight of oxygen

ACKNOWLEDGEMENT:

Portions of this outline were taken from outlines prepared by R. J. Lishka and Charles R. Feldmann.

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This outline was prepared by Audrey D. Kroner, Chemist, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Chemical Analysis, Chemical Oxygen Demand, Organic Compounds, Oxidation, Oxygen, Oxygen Demand, Oxygen Requirements, Water Analysis

DATA SHEET

ml of $K_2Cr_2O_7$ used to standardize the ferrous ammonium sulfate (FAS) = 10.0

N of $K_2Cr_2O_7$ = 0.25

ml of FAS used to titrate the $K_2Cr_2O_7$ = ________

$$\text{N FAS} = \frac{10.0 \times 0.25}{\text{ml FAS}}$$

$$= \frac{2.5}{\quad}$$

$$= \underline{\qquad\qquad}$$

ml FAS used for blank, a = ________

ml FAS used for sample, b = ________

ml sample = ________

$$\text{COD, in mg liter} = \frac{(a - b) \times \text{N FAS} \times 8 \times 1000}{\text{ml sample}}$$

$$= \frac{(\quad - \quad) \times \quad \times 8 \times 1000}{\quad}$$

$$= \underline{\qquad\qquad}$$

PHOSPHORUS IN THE AQUEOUS ENVIRONMENT

I Phosphorus is closely associated with water quality because of (a) its role in aquatic productivity such as algal blooms, (b) its sequestering action, which causes interference in coagulation, (c) the difficulty of removing phosphorus from water to some desirable low concentration, and (d) its characteristic of converting from one to another of many possible forms.

A Phosphorus is one of the primary nutrients such as hydrogen (H), carbon (C), nitrogen (N), sulfur (S) and phosphorus (P).

1 Phosphorus is unique among nutrients in that its oxidation does not contribute significant energy because it commonly exists in oxidized form.

2 Phosphorus is intimately involved in oxidative energy release from and synthesis of other nutrients into cell mass via:

a Transport of nutrients across membranes into cell protoplasm is likely to include phosphorylation.

b The release of energy for metabolic purposes is likely to include a triphosphate exchange mechanism.

B Most natural waters contain relatively low levels of P (0.01 to 0.05 mg/L) in the soluble state during periods of significant productivity.

1 Metabolic activity tends to convert soluble P into cell mass (organic P) as a part of the protoplasm, intermediate products, or sorbed material.

2 Degradation of cell mass and incidental P compounds results in a feedback of lysed P to the water at rates governed by the type of P and the environment. Aquatic metabolic kinetics show marked influences of this feedback.

3 The concentrations of P in hydrosoils, sludges, treatment plant samples and soils may range from 10^2 to 10^6 times that in stabilized surface water. Both concentration and interfering components affect applicability of analytical techniques.

II The primary source of phosphorus in the aqueous system is of geological origin. Indirect sources are the processed mineral products for use in agriculture, household, industry or other activities.

A Agricultural fertilizer run-off is related to chemicals applied, farming practice and soil exchange capacity.

B Wastewaters primarily of domestic origin contain major amounts of P from:

1 Human, animal and plant residues

2 Surfactants (cleaning agent) discharge

3 Microbial and other cell masses

C Wastewaters primarily of industrial origin contain P related to:

1 Corrosion control

2 Scale control additives

3 Surfactants or dispersants

4 Chemical processing of materials including P

5 Liquors from clean-up operations of dusts, fumes, stack gases, or other discharges

III Phosphorus terminology is commonly confused because of the interrelations among biological, chemical, engineering, physical, and analytical factors.

A Biologically, phosphorus may be available as a nutrient, synthesized into living mass, stored in living or dead cells, agglomerates, or mineral complexes, or converted to degraded materials.

B Chemically, P exists in several mineral and organic forms that may be converted from one to another under favorable conditions. Analytical estimates are based upon physical or chemical techniques necessary to convert various forms of P into ortho phosphates which alone can be quantitated in terms of the molybdenum blue colorimetric test.

C Engineering interest in phosphorus is related to the prediction, treatment, or control of aqueous systems to favor acceptable water quality objectives. Phosphorus removal is associated with solids removal.

D Solubility and temperature are major physical factors in phosphorus behavior. Soluble P is much more available than insoluble P for chemical or biological transformations and the rate of conversion from one to another is strongly influenced by temperature.

E Table 1 includes a classification of the four main types of chemical P and some of the relationships controlling solubility of each group. It is apparent that no clear-cut separation can be made on a solubility basis as molecular weight, substituent and other factors affect solubility.

F Table 2 includes a scheme of analytical differentiation of various forms of P based upon:

1 The technique required to convert an unknown variety of phosphorus into ortho P which is the only one quantitated by the colorimetric test.

2 Solubility characteristics of the sample P or more precisely the means required to clarify the sample.

a Any clarification method is subject to incomplete separation. Therefore, it is essential to specify the method used to interpret the yield factor of the separation technique. The degree of separation of solubles and insolubles will be significantly different for:

1 Membrane filter separation (0.5 micrometer pore size)

2 Centrifugation (at some specified rpm and time)

3 Paper filtration (specify paper identification)

4 Subsidence (specify time and conditions)

G Analytical separations (Table 2) like those in Table 1, do not give a precise separation of the various forms of P which may be included quantitatively with ortho or poly P. Conversely some of the poly and organic P will be included with ortho P if they have been partially hydrolyzed during storage or analysis. Insolubles may likewise be included as a result of poor separation and analytical conditions.

1 The separation methods provide an operational type of definition adequate in most situations if the "operation" is known. Table 2 indicates the nature of incidental P that may appear along with the type sought.

Table 1

PHOSPHORUS COMPOUNDS CLASSIFIED BY CHEMICAL AND SOLUBILITY RELATIONS

Form	Water Soluble(1)	Insoluble(1)
1. Ortho phosphates $(PO_4)^{-3}$	Combined with monovalent cations such as H^+, Na^+, K^+, NH_4^+	Combined with multi valent cations such as Ca^{+2} Al^{+3} Fe^{+3}
2. Poly phosphates $(P_2O_7)^{-4}$, $(P_3O_{10})^{-5}$ $(P_3O_9)^{-3}$ and others depending upon the degree of dehydration.	as in 1 above Increasing dehydration decreases solubility	(a) as in 1 above (b) multi P polyphosphates (high mol. wt.) including the "glassy" phosphates
3. Organic phosphorus R-P, R-P-R (2) (unusually varied nature)	(a) certain chemicals (b) degradation products (c) enzyme P (d) phosphorylated nutrients	(a) certain chemicals (b) cell mass, may be colloidal or agglomerated (c) soluble P sorbed by insoluble residues
4. Mineral phosphorus	(a) as in 1 above	(a) as in 1 above (b) as in 2 above (c) geological P such as phosphosilicates (d) certain mineral complexes.

(1) Used in reference to predominance under common conditions.

(2) R represents an organic radical, P represents P, PO_4^{-3}, or its derivatives.

---

2 Total P in Table 2 includes liquid and separated residue P that may exist in the whole sample including silt, organic sludge, or hydrosoils. This recognizes that the feedback of soluble P from deposited or suspended material has a real effect upon the kinetics of the aqueous environment.

Table 2

PHOSPHORUS COMPOUNDS CLASSIFIED BY ANALYTICAL METHODOLOGY

Desired P Components	Technique(1)	Incidental P Included(2)
1. Ortho phosphates	No treatment on clear samples	Easily hydrolyzed (a) poly phosphates - (b) organic -P, - (c) Mineral -P, + or -
2. Polyphosphates (2)-(1) = poly P (hydrolyzable)	acid hydrolysis on clear samples, dilute (a) H_2SO_4 (b) HCl heated	(a) ortho-P + (b) organic -P + or - (c) mineral -P + or -
3. Organic phosphorus (3) - (2) + org P (hydrolyzable)	acid + oxidizing hydrolysis on whole sample. dilute (a) $H_2SO_4 + HNO_3$ (b) $H_2SO_4 + (NH_4)_2S_2O_8$ heated	(a) ortho P + (b) poly P + (c) mineral P + or -
4. Soluble phosphorus (preferably classified by clarification method)	clarified liquid following filtration, centrifugation or subsidence	generally includes (a) 1, 2, or 3 (b) particulates not completely separated
5. Insoluble phosphorus (residue from clarification)	Retained residues separated during clarification See (6)	(a) generally includes sorbed or complexed solubles.
6. Total phosphorus	Strong acid + oxidant digestion (a) $H_2SO_4 + HNO_3$ (b) $H_2SO_4 + HNO_3 + HClO_4$ (c) $H_2O_2 + Mg(NO_3)_2$ fusion	all components in 1, 2, 3, 4, 5 in the whole sample

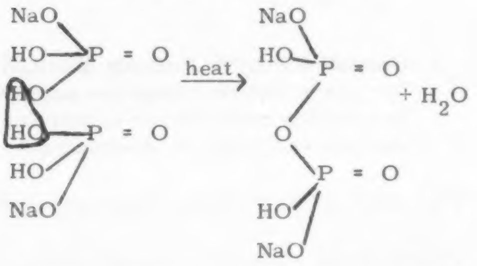
(1) Determinative step by phospho molybdate colorimetric method.

(2) Coding: + quantitative yield
- a small fraction of the amount present
+ or - depends upon the individual chemical and sample history

IV Polyphosphates are of major interest in cleaning agent formulation, as dispersants, and for corrosion control.

A They are prepared by dehydration of ortho phosphates to form products having two or more phosphate derivatives per molecule.

1 The simplest polyphosphate may be prepared as follows:



mono sodium ortho phosphate (2) disodium dihydrogen polyphosphate

2 The general form for producing polyphosphates from mono substituted orthophosphates is:

$$n\ (NaH_2PO_4) \xrightarrow[200-400^\circ C]{heat} (NaPO_3)_n + n\ H_2O$$

3 Di-substituted ortho phosphates or mixtures of substituted ortho phosphates lead to other polyphosphates:

$$2Na_2HPO_4 + NaH_2PO_4 \xrightarrow{heat} Na_5P_3O_{10} + 2H_2O$$

disodium hydrogen ortho phosphate + mono sodium di hydrogen ortho phosphate → pentasodium tri-phosphate + water

4 The polyphosphate series usually consist of the polyphosphate anion with a negative charge of 2 to 5. Hydrogen or metals commonly occupy these sites. The polyphosphate can be further dehydrated by heat as long as hydrogen remains. Di or trivalent cations generally produce a more insoluble polyphosphate than the same cation in the form of insoluble ortho phosphate. Insolubility increases with the number of P atoms in the polyphosphate. The "glassy" polyphosphates are a special group with limited solubility that are used to aid corrosion resistance in pipe distribution systems and similar uses.

B Polyphosphates tend to hydrolyze or "revert" to the ortho P form by addition of water. This occurs whenever polyphosphates are found in the aqueous environment.

1 The major factors affecting the rate of reversion of poly to orthophosphates include:

a) Temperature, increased T increases rate

b) pH, lower pH increases rate

c) Enzymes, hydrolase enzymes increase rate

d) Colloidal gels, increase rate

e) Complexing cations and ionic concentration increase rate

f) Concentration of the polyphosphate increases rate

2 Items a, b and c have a large effect upon reversion rate compared with other factors listed. The actual reversion rate is a combination of listed items and other conditions or characteristics.

3 The differences among ortho and ortho + polyphosphates commonly are close to experimental error of the colorimetric test in stabilized surface water samples. A significant difference generally indicates that the sample was obtained relatively close to a source of polyphosphates and was promptly analyzed. This implies that the reversion rate of polyphosphates is much higher than generally believed.

V SAMPLING AND PRESERVATION TECHNIQUES

A Sampling

1 Great care should be exercised to exclude any benthic deposits from water samples.

2 Containers should be cleaned in the same manner as labware (see VI. B. 1. below).

3 Certain plastic containers may be used. Possible adsorption of low concentrations of phosphorus should be checked.

4 If a differentiation of phosphorus forms is to be made, filtration should be carried out immediately upon sample collection. A membrane filter of 0.45 µm pore size is recommended for reproducible separations.

B Preservation

1 If at all possible, samples should be analyzed on the day of collection. At best, preservation measures only retard possible changes in the sample.

a Possible physical changes include solubilization, precipitation, absorption on or desorption from suspended matter.

b Possible chemical changes include reversion of poly to ortho P and decomposition of organic or mineral P.

c Possible biological changes include microbial decomposition of organic P and algal or bacterial growth forming organic P.

2 If it is impossible to do total phosphorus determinations on the day of collection, refrigerate at 4°C and add 2 ml concentrated H_2SO_4 per liter. Limit of holding for samples thus preserved is 24 hours. (6)

a Refrigeration decreases hydrolysis and reaction rates and also losses due to volatility.

b Sulfuric acid limits biological changes.

c Mercuric chloride also limits biological changes, but interferes with the analytical procedure if the chloride level is less than 50 mg Cl/liter. (See VI B. 2. below). Disposal of mercury-containing test wastes also adds time to the procedure.

3 Consult the EPA Methods Manual(6) for preservation measures applicable to samples collected to determine various fractions of phosphorus.

VI THE EPA ANALYTICAL PROCEDURE(6)

A This is a colorimetric determination, specific for orthophosphate. Depending on the nature of the sample and on the type of data sought, the procedure involves two general operations:

1 Conversion of phosphorus forms to soluble orthophosphate (See Fig. 1).

a sulfuric acid-hydrolysis for polyphosphates, and some organic P compounds,

b persulfate digestion for organic P compounds

2 The color determination involves reacting dilute solutions of phosphorus with ammonium molybdate and potassium antimonyl tartrate in an acid medium to form an antimony-phosphomolybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the orthophosphate concentration.

Color absorbance is measured at 880 nm or 650 nm and a concentration value obtained using a standard curve.

Reagent preparation and the detailed procedure can be found in the EPA manual.

The methods described there are usable in the 0.01 to 0.5 mg/liter phosphorus range. This range can be extended by dilution of samples.

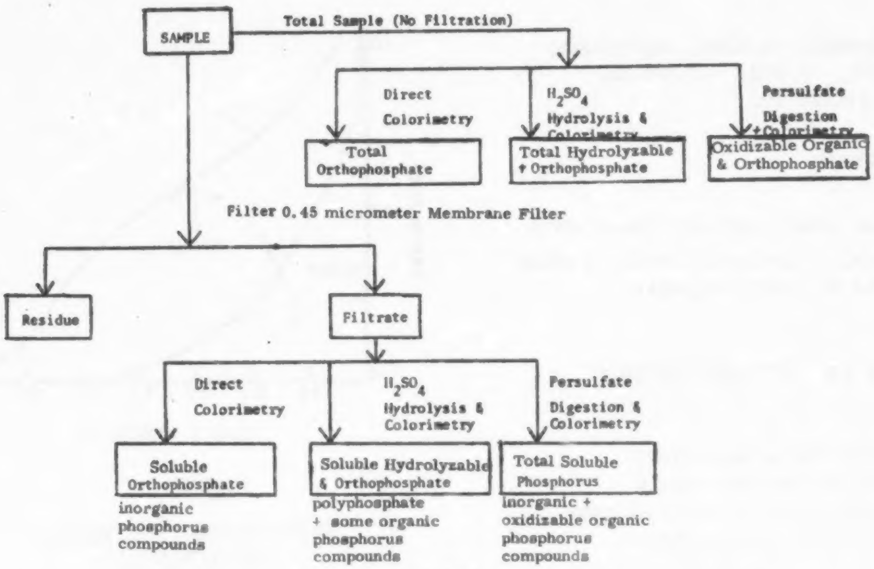

SAMPLE
Total Sample (No Filtration)
Direct Colorimetry
H2SO4 Hydrolysis & Colorimetry
Persulfate Digestion + Colorimetry
Total Orthophosphate
Total Hydrolyzable + Orthophosphate
Oxidizable Organic & Orthophosphate
Filter 0.45 micrometer Membrane Filter
Residue
Filtrate
Direct Colorimetry
H2SO4 Hydrolysis & Colorimetry
Persulfate Digestion & Colorimetry
Soluble Orthophosphate
Soluble Hydrolyzable & Orthophosphate
Total Soluble Phosphorus
inorganic phosphorus compounds
polyphosphate + some organic phosphorus compounds
inorganic + oxidizable organic phosphorus compounds

FIGURE 1
ANALYTICAL SCHEME FOR DIFFERENTIATION OF PHOSPHORUS FORMS

B Interferences

1 Erroneous results from contaminated glassware is avoided by cleaning it with hot 1:1 HCl, treating it with procedure reagents and rinsings with distilled water. Preferably this glassware should be used only for the determination of phosphorus and protected from dust during storage. Commercial detergents should never be used.

2 Low total phosphorus values have been reported because of possible adsorption of phosphorus on iron, aluminum, manganese or other metal precipitates formed in wastewater samples.

a After digestion, adjust the pH of the sample to 7.0 ± 0.2 with 1N NaOH using a pH meter. If sample is not clear, add 2-3 drops 11N H_2SO_4 and filter.

b Filter through phosphorus-free 0.45 micrometer pore size cellulose filter. See Standard Methods, (7) page 472 about washing filters before use, since the discs can introduce significant phosphorus contamination.

3 The total phosphorus procedure requires a pH adjustment with a pH meter. Buffers made with phosphates are used to calibrate the meter in the applicable range. The meter electrodes must be thoroughly flushed free of buffer before their use with phosphorus test solutions. Otherwise, significant phosphorus contamination will result.

4 Others have reported interference from arsenic, arsenates, chlorine, chromium, sulfides, nitrite, tannins, lignin and other minerals and organics at high concentrations.

C Precision and Accuracy(6)

1 Organic phosphate - 33 analysts in 19 laboratories analyzed natural water samples containing exact increments of organic phosphate of 0.110, 0.132, 0.772, and 0.882 mg P/liter.

Standard deviations obtained were 0.033, 0.051, 0.130 and 0.128 respectively.

Accuracy results as bias, mg P/liter were: + 0.003, + 0.016, + 0.023 and - 0.008, respectively.

2 Orthophosphate was determined by 26 analysts in 16 laboratories using samples containing orthophosphate in amounts of 0.029, 0.038, 0.335 and 0.383 mg P/liter.

Standard deviations obtained were 0.010, 0.008, 0.018 and 0.023 respectively.

Accuracy results as bias, mg P/liter were -0.001, -0.002, -0.009 and -0.007 respectively.

D Automated Methods

The EPA Manual also contains a procedure for the automated colorimetry method using ascorbic acid as the reducing agent.

VII VARIABLES IN THE COLORIMETRIC PROCEDURE

Several important variables affect formation of the yellow heteropoly acid and its reduced form, molybdenum blue, in the colorimetric test for P.

A Acid Concentration during color development is critical. Figure 2 shows that color will appear in a sample containing no phosphate if the acid concentration is low. Interfering color is negligible when the normality with respect to H_2SO_4 approaches 0.4.

1 Acid normality during color development of 0.3 to slightly more than 0.4 is feasible for use. It is preferable to control acidity carefully and to seek a normality closer to the higher limits of the acceptable range.

2 It is essential to add the acid and molybdate as **one solution.**

3 The aliquot of sample must be neutralized prior to adding the color reagent.

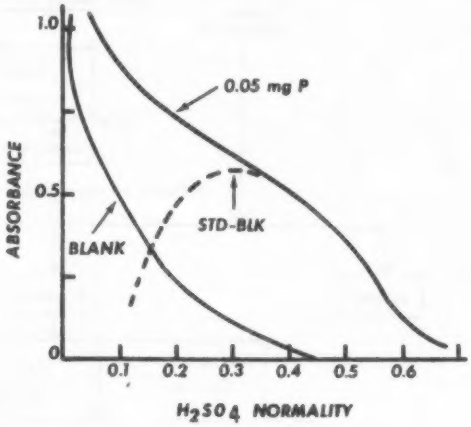


Figure 2

0-PHOSPHATE COLOR
VS ACIDITY

B Choice of Reductant - Reagent stability, effective reduction and freedom from deleterious side effects are the bases for reductant selection. Several reductants have been used effectively. Ascorbic acid reduction is highly effective in both marine and fresh water. It is the reductant specified in the manual NPDES procedure.

C Temperature affects the rate of color formation. Blank, standards, and samples must be adjusted to the same temperature (± 1°C), (preferably room temperature), before addition of the acid molybdate reagent.

D Time for Color Development must be specified and consistent. After addition of reductant, the blue color develops rapidly for 10 minutes then fades gradually after 12 minutes.

VIII DETERMINATION OF TOTAL PHOSPHORUS

A Determination of total phosphorus content requires the acid-hydrolysis and persulfate treatments to convert all phosphorus forms to the test-sensitive orthophosphate form.

B Determining total phosphorus content yields the most meaningful data since the various forms of phosphorus may change from one form to another in a short period of time. (See part V, B1)

IX DEVELOPMENT OF A STANDARD PROCEDURE

Phosphorus analysis received intensive investigation; coordination and validation of methods is more difficult than changing technique.

A Part of the problem in methods arose because of changes in analytical objectives such as:

1 Methods suitable to gather "survey" information may not be adequate for "standards".

2 Methods acceptable for water are not necessarily effective in the presence of significant mineral and organic interference characteristic of hydrosoils, marine samples, organic sludges and benthic deposits.

3 Interest has been centered on "fresh" water. It was essential to extend them for marine waters.

4 Instrumentation and automation have required adaptation of methodology.

B Analysts have tended to work on their own special problems. If the method apparently served their situations, it was used. Each has a "favorite" scheme that may be quite effective but progress toward widespread application of "one" method has been slow. Consequently, many methods are available. Reagent acidity, Mo content, reductant and separation techniques are the major variables.

C The persulfate digestion and single reagent (ascorbic acid) method described in VI above is the only method currently listed in the Federal Register "List of Approved Procedures" for NPDES requirements.

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This outline was prepared by Ferdinand J. Ludzack, former Chemist, National Training Center, and by Audrey D. Kroner, Chemist, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Chemical Analysis, Nutrients, Phosphates, Phosphorus, Phosphorus Compounds, Pollutant Identification, Sampling, Water Analysis, Water Pollution Sources.

USE OF A SPECTROPHOTOMETER

I SCOPE AND APPLICATION

A Colorimetry

Many water quality tests depend on a treating a series of calibration standard solutions which contain known concentrations of a constituent of interest, and also the sample(s) with reagents to produce a colored solution. The greater the concentration of the constituent, the more intense will be the resulting color. A spectrophotometer is used to measure the amount of light of appropriate wavelength which is absorbed by equal "thicknesses" of the solutions. Results from the standards are used to construct a calibration (standard) curve. Then the absorbance value for the sample is located on the curve to determine the corresponding concentration.

B Lambert Beer Law

States the applicable relationships:

$$A = e\,b\,c$$

1 A = absorbance

2 e = molar absorptivity

3 b = light path in cm

4 c = concentration in moles/liter

II APPARATUS

A Requirements

Are given as part of the method write-up

1 The applicable wavelength is specified. The unit used is nanometers (nm).

2 The light path (cell dimension) is often open-ended, e.g., "one cm or longer." One must know the light path length in the available spectrophotometer, because it is inversely related to the usable concentrations in the test. (Longer path lengths detect lower concentrations).

3 NOTE: For National Pollutant Discharge Elimination System (permit), or for Drinking Water Regulations test requirements, check with the issuing/report agency before using light paths (cells) that differ from the length specified in the approved method. If you have permission to use an alternate path length, concentrations for the test can be adjusted accordingly. These adjustments are discussed in IV and in VII (below).

III PREPARATION OF THE SPECTROPHOTOMETER

A Phototube/Filter

1 May have to choose a phototube for use above or below a particular wavelength.

2 A filter may be required.

3 If the available instrument involves a choice, check that the phototube (and filter, if applicable,) required for the wavelength to be used is in the instrument.

4 Always handle and wipe off the phototube and/or filter with tissue to avoid leaving fingerprints.

B Cell compartment

1 This area must be kept clean and dry at all times.

C Cells

1 A set must "match" each other in optical properties. To check this, use the same solution at the same wavelength, and verify that the absorbance value is the same for each cell.

2 Alternatively, a single cell can be used if it is thoroughly rinsed after each reading.

3 Instrument cells should be free of scratches and scrupulously clean.

  a Use detergents, organic solvents or 1:1 nitric acid-water.

  b Caustic cleaning compounds might etch the cells.

  c Dichromate solutions are not recommended because of adsorption possibilities.

  d Rinse with tap, then distilled water.

  e A final rinsing and drying with alcohol or acetone before storage is a preferred practice.

D Warm-Up

1 Plug in the power cord.

2 Turn the power switch on and give it an additional half-turn to keep the needle from "pegging."

3 Wait to use until the recommended warm-up time has passed. Anywhere from 10 to 30 minutes may be required.

4 If the instrument drifts during zeroing, allow a longer time.

E Wavelength Alignment

One reference is the known, maximum absorption for a dilute solution of potassium permanganate which has a dual peak at 526 nm and 546 nm. Use 2 matched cells for the following steps:

1 Prepare a dilute solution of potassium permanganate (about 10 mg/L).

2 Follow the steps in VI A, Zeroing Operation, using a wavelength of 510 nm, and distilled water as a "reagent blank." Keep the water in the cell during this entire procedure.

3 Rinse the matched cell two times with tap water, then two times with the permanganate solution.

4 Fill the cell three-fourths full with the permanganate solution. Keep the permanganate solution in this cell during this entire procedure.

5 Thoroughly wipe the cell with a tissue. Hold the cell by the top edges.

6 Open the cover and gently insert the cell, aligning it to the ridge as before.

7 Close the cover.

8 Record the wavelength and the absorbance reading on a sheet of paper.

9 Remove the cell of permanganate solution and close the cover.

10 Set the wavelength control at the next graduation (+ 5nm).

11 If the needle is not at infinite (symbol ∞) absorbance, use the left knob to re-set it.

12 Insert the cell containing distilled water using the techniques noted in 5, 6 and 7 above.

13 If necessary, use the right knob to re-set the needle at zero absorbance.

14 Remove the cell and insert the cell of permanganate solution using the techniques noted in 5, 6 and 7 above.

15 Record the wavelength and the absorbance reading.

16 Repeat steps 9 through 15 above until absorbance readings are recorded at 5 nm increments from 510 nm through 560 nm.

17 Make a graph plotting absorbance readings against wavelengths. With very good resolution, there will be two peaks - one at 525 nm and one at 545 nm. A single flat topped "peak" between these two wavelengths is acceptable.

18 If the maximum absorbances [peak(s)] occur below or above 526 nm or 546 nm, and at a number of scale units different from the stated instrument accuracy, the wavelength control is misaligned. To compensate for this until the instrument can be serviced, add or subtract the error scale units when setting wavelengths for subsequent tests.

IV CALIBRATION STANDARDS

A Requirements

A set of calibration standards is required, with concentrations usable in the available spectrophotometer cell (light path length).

1 The method reference may provide a table of light path lengths and the corresponding applicable concentration range for calibration standards, so one can choose the range required for his instrument cell or sample concentration.

2 The method reference may give directions for preparing one range of concentrations for a given light path length. If your cell provides a different length, your concentration requirements can be easily calculated by recalling that the light path length is inversely related to concentration. Thus, if your cell is twice the given path length, you need the given concentrations divided by two.

3 The method reference may give directions for preparing only one range of concentrations for the calibration standards, and then not be specific about the associated path length. You will have to test if the range is applicable to your instrument by preparing the given concentrations, obtaining absorbance values for them and checking the results according to section VII (below).

B Preparation

The calibration standards required for spectrophotometric measurements are so dilute, that they are commonly prepared by diluting stronger solutions. These are described in general terms below. Weights and volumes involved in preparing these solutions for a specific test can be found in the method write-up.

1 Stock Solutions

a Prepare by weighing or measuring a small amount of a chemical containing the constituent of interest and dissolving it to a one liter volume.

b Common stock solutions have concentrations in the range of 0.1 to 1.0 mg/ml.

c Most are refrigerated for storage and some are further treated by adding a preservative. Many are stable up to six months.

2 Standard Solutions

a Prepare by diluting a stock solution (at room temperature). Common volumes are 10.0 or 20.0 ml of stock diluted to one liter.

b Resulting standard solutions have concentrations in the range of 1.0 to 10.0 ug/ml.

c Although some standard solutions may be stable for a period of time, it is a recommended practice to prepare them on the day of use.

3 Calibration (Working) Standard Solutions

a Prepare by diluting a standard solution. Usually a set of calibration standards is required so that resulting concentrations give five to seven results within the sensitivity limits of the instrument. Common volumes are 1 to 10 ml of standard solution diluted to 100 ml.

b Resulting solutions might have concentrations in the range of 0.01 and 1.0 ug/ml.

c A reagent blank (distilled water) should be included in the set of standards.

4 Adjusting Concentrations

a You may find it necessary to adjust preparation quantities given in the method write-up, because your cell (light path length) differs from the example.

b These adjustments are discussed in A Requirements (above), and are usually applied to the Standard (intermediate) Solution.

C Chemical Treatment

1 Most colorimetric methods require that the calibration standards (including the reagent blank) are to be treated as the sample. Thus, they are to be processed through pretreatments and through the test as if they were samples. Then any test effects on sample results will be compensated by the same effects on results obtained for the treated standards.

2 One should be aware that pH is a critical condition for most colorimetric reactions. Ordinarily, a pH adjustment is included in the test method and reagents include chemicals to control pH. Thus, the processed standards correspond to the samples regarding pH, and thus they correspond in degree of color development. If standards are processed in some other manner, they must be pH adjusted to correspond to the samples at the time of color development.

V SAMPLE DILUTIONS

A Concentration Limits

The concentration of the sample must result in an absorbance within the range of the calibration standards, i.e., accurately detectable in the light path provided by the instrument. A dilution before analysis may be required to accomplish this. It is not accurate to dilute a sample after processing in order to obtain a usable absorbance reading.

1 Record dilution volumes so a dilution factor can be calculated and applied to results.

2 An analyst often has a good estimate of the expected concentration of a sample if s/he routinely tests samples from the same source. In this case, a single dilution, if any, is usually sufficient.

3 If a sample is from an unknown source, the analyst has several choices.

a Process the sample. If the reading shows it is too concentrated, dilute it until you get a value in the usable range. This result is not accurate enough to report, but you now know how to dilute the sample to process it through the test to get usable results.

b Prepare at least a 50% dilution and analyze it plus an undiluted aliquot.

c Prepare a variety of dilutions.

d Use some other analytical method to get a rough estimate of the expected concentration.

B Final Volumes

1 Dilute to a final volume sufficient to rinse the measuring glassware and provide the test volume cited in the referenced method.

2 Save any undiluted sample.

VI PROCEDURE FOR USING A SPECTROPHOTOMETER

A Zeroing Operation

The following steps have been written for spectrophotometers used in this course. Check the manual for the available instrument for the steps applicable for your work.

1 Set the wavelength control to the setting specified for the standards you are testing. Approach the setting by beginning below the number and dialing up to it.

2 If a cell is in the holder, remove it.

3 Close the cell holder cover.

4 Turn the power switch/zero control (left) knob until the needle reads infinite (symbol ∞) absorbance (on the lower scale).

5 Rinse a cell two times with tap water, two times with distilled water, then two times with the reagent blank solution.

6 Fill the cell about three-fourths full with reagent blank solution.

7 Thoroughly wipe the outside of the cell with a tissue to remove fingerprints and any spilled solution. Hold the cell by the top edges.

8 Open the cell holder cover and gently slide the cell down into the sample holder.

9 Slowly rotate the cell until the white vertical line on the cell is in line with the ridge on the edge of the sample holder.

10 Close the cover and turn the light control (right) knob until the needle reads zero absorbance (on the lower scale).

11 Record an absorbance of zero for this zero concentration solution on a data sheet. (See next page).

12 Slowly remove the cell and close the cover. (No solution should spill inside the instrument). Keep the solution in the cell.

13 The needle should return to the infinite absorbance setting. It it does not:

- a Reset the needle to the ∞ absorbance mark using the power switch/zero (left) control knob.
- b Re-test the reagent blank solution using steps 7 through 12 above.
- c If the needle does not return to the ∞ absorbance mark, another setting as noted in a. and b. is required. Additional warm-up time may be necessary before these settings can be made.

B Reading Absorbances

Using a single cell in the spectrophotometers used in this course

1 Discard any solution in the cell.

2 Rinse the cell two times with tap water, and two times with distilled water. Then rinse it two times with the lowest concentration standard remaining to be tested, or with processed sample.

3 Fill the cell about three-fourths full with the same standard or sample.

4 Use a tissue to remove any fingerprints from the cell and any droplets on the outside. Hold the cell by the top edges.

5 Open the cell holder cover and gently slide the cell down into the sample holder.

6 Slowly rotate the cell until the white vertical line on the cell is in line with the ridge on the edge of the sample holder.

7 Close the cover.

8 Record the concentration of the standard and its absorbance on a data sheet. (For a sample, record its identification code and its absorbance on the data sheet).

DATA SHEET

Concentration mg/liter	Absorbance
0.00	
SAMPLE	
SAMPLE	

9 Repeat steps 1 through 8 (above) for each standard and sample to be tested. If a large number of measurements are to be made, check the instrument calibration every fifth reading.

a Use another aliquot of a solution already tested to see if the same reading is obtained. If not, repeat the zeroing operation in A (above).

b Alternatively, you can use the blank, if supply permits, and repeat the zeroing operation in A. (above).

10 When all the readings have been obtained, discard any solution remaining in the cell and rinse the cell with tap water. Clean the cell more thoroughly, (III C. 3), as soon as possible.

11 If no other tests are to be done, turn off the instrument, pull out the plug and replace any protective covering.

VII CHECKING RESULTS

A Readings Greater Than 0.70

On our instrument, these are considered to be inaccurate. Check the manual for your instrument or check the scale divisions to determine the limit for other models.

1 Do not use readings greater than 0.70 to develop a calibration curve.

2 From five to eight points (counting zero) are recommended for constructing a calibration curve. If you have fewer than five usable values, you should not draw a curve.

3 To prevent excessively high values in future tests, decrease the cell path length, if possible, by using an adapter and smaller cell.

4 If you cannot decrease the cell path length, you can at least obtain enough values to construct a curve. Prepare standards with five to eight concentrations ranging from zero to the concentration of the standard having an absorbance nearest to 0.70. This gives you more values for a curve, but it reduces the applicable range of the test. Usually the sample can be diluted before testing so the result will fit on the standard curve.

B Highest Reading is Less Than 0.6

1 Increase the cell path length by using a larger cell. A higher reading results.

2 Prepare a different set of standards with greater concentrations.

VIII CONSTRUCTING A CALIBRATION CURVE

If you have from five to eight usable absorbance values, you can construct a concentration curve.

A Graph Paper

Should be divided into squares of equal size in both directions

B Concentration Axis

1 Labeling

The longer side should be labeled at equal intervals with the concentrations of the calibration standards marked from 0.0 to at least the highest concentration recorded for the standards on the data sheet.

2 Units

a It is most convenient to express these concentrations in the units to be reported. Otherwise, a unit-conversion factor would have to be applied to obtain final, reportable values every time you use the curve.

b Example: If you dilute a standard solution to make 100.0 ml volumes of calibration standards, you have a choice in expressing the resulting concentrations. You can use weight/100 ml, or you can calculate weight/1 liter. If you are to report results as weight/liter, but you construct your curve using weight/100 ml, you will have to multiply every sample result from the curve by $\frac{1000}{100}$ or 10 to obtain the reportable value. It is much easier to convert the original calibration standard concentrations to the desired units and to use these as labels on the graph.

C Absorbance Axis

1 Labeling

The shorter side should be labeled at equal intervals with absorbance numbers marked from 0.00 to at least 0.70 absorbance units.

D Plotting the Curve

1 Use the absorbances recorded for each standard concentration to plot points for the curve.

2 The points should fall in a reasonably straight line.

3 Use a straight-edge to draw a line of best fit through the points. If the points do not all fall on the line, an acceptable result is an equal number of points falling closely above, as well as below the line. Experience provides a basis for judging acceptability.

4 It is not permissable to extrapolate the curve.

IX USING THE CALIBRATION CURVE

A Finding concentration of the sample

1 Use the absorbance value(s) recorded for the sample(s), and the calibration curve to find the concentration(s). If the concentration units differ from those required for reporting results, apply a unit conversion factor (VIIIB. 2).

2 If more than one dilution of a sample was tested, use the result that falls nearest the middle of the curve.

B If a sample was diluted, calculate the dilution factor and apply it to the concentration you find for the sample from the calibration curve.

1 **Dilution Factor=**

$$\frac{\text{final dilution volume}}{\text{ml sample used in dilution}}$$

2 **Example:** You diluted 10 ml sample to 50 ml. The concentration found by using a calibration curve was 0.5 mg/liter.

Then:

$$\text{constituent, mg/l} = \frac{50\text{ml}}{10\text{ ml}} \times \frac{0.5\text{ mg}}{\text{liter}}$$

$$= 2.5\text{ mg/liter}$$

This outline was prepared by Audrey D. Kroner, Chemist, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268

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CHEMICAL TESTS,OBSERVATIONS,AND MEASUREMENTS IN THE FIELD

I INTRODUCTION

A Laboratory determinations with approved equipment and convenient facilities by experienced specialists generally are easier, faster, and more reliable. It is advisable to transport the samples to the laboratory whenever it is feasible to do so.

B Field tests are essential because:

1 Certain sample components are inherently unstable with respect to biological, chemical, or physical changes. Any test result performed in the laboratory may not represent true conditions on site at the time because of delay, displacement, or changed conditions.

2 Subsequent operations generally may be coordinated and made more meaningful by preliminary on site field investigation to identify and evaluate problems, locate critical areas, and minimize surprises.

II FIELD TEST CONDITIONAL CONSIDERATIONS

A Moving the laboratory into the field means improvisation and adaptation to more "primitive" conditions.

1 Rugged construction of field equipment is a first consideration. Sturdy and convenient cases are required; the case often may be the only available workbench. Polyethylene bottles, beakers, burets, and pipets generally are necessary to eliminate breakage of fragile glass.

2 Portability is essential, particularly when the site is not accessible by boat or car. Ideally, the field kit should be small enough and light enough to be carried by one person for extended periods.

3 Procedures for field use are restricted in equipment and manipulation. In general, it is not possible to use long, tedious routines or highly precise measurements. Numerous reagents generally cannot be carried. Quick positive reactions are essential. Small visual comparators, test papers or spot tests are popular. Titration assemblies must be modified for field use.

4 Instrumentation increases objectivity of the measurements but the instruments must be adapted to the items outlined in II. A. 1, 2 and 3.

The instruments commonly used must be simplified yet rugged enough to keep working in spite of temperature changes, moisture, bumps, etc. A meaningful validation procedure must be worked out for calibration on site with simple adjustment or repair for emergencies.

5 Personnel engaged in field testing often are unaccustomed to laboratory procedures. Advance training is essential in procedures and instrumentation used. Field testing is a multi-disciplinarian operation:

a The individuals must be good observers and recognize what may be significant later.

b They must be good interpreters of a variety of observed and derived test information.

c They must be good technicians to follow prescribed procedures diligently and recognize anomalous behavior when it occurs.

d They must be good reporters to describe what happened, where it happened, and when. Failure to report anomalous behavior and related events in an understandable and consistent fashion may render the entire effort meaningless because of some doubt or inconsistency. Subsequent operations also may be mislead by the early observer.

B Use of field data imposes certain constraints relatable to the item, the methods, and to the personnel involved:

1 How valid is it?

2 How consistent is it in line with other observed or recorded information?

3 What backup information is required to verify suggested information?

C Decisions must be made on all field data applications:

1 Where "in-place" and "now" measurements are required how much of the laboratory must be moved out to the site to obtain data reliable enough to meet objectives?

2 Which items are to be determined on site? Which items in a central laboratory?

3 What use is to be made of the preliminary field estimates?

4 What do you "need" to know?

5 What would be "nice" to know?

6 In line with facilities, time, cost, manpower and objectives, what can you determine?

III COMPONENT CHARACTERISTICS FAVORING FIELD TESTING:

A Any item that changes rapidly during a holding period as a result of temperature or pressure variations, or is highly reactive from a biological, chemical, or physical standpoint, usually means that it must be determined on site and in place with minimum time lapse and manipulation.

1 Dissolved gases, such as O_2, CO_2, H_2S, Cl_2, are sensitive to pressure or temperature changes and may react with other sample components readily. These require in place and now readout.

2 The collective analysis of all forms of a given item may be preserved and analyzed later. Estimates of different forms of the same substance are not subject to delay unless they can be separated promptly. Examples of this include the relative ratios of oxidized or reduced substances, such as Cu^{+1} and Cu^{+2}, Fe^{+2} and Fe^{+3}, Cr^{+3} and Cr^{+6}; hydrolysis is the principal factor in the ratios of organic and NH_3-N and in changes among organic, poly and ortho-P.

3 Reactive substances contributing to "oxygen demand" tests, such as BOD or other respiratory tests generally require a minimum time delay before testing and are not amenable to preservation without altering results.

4 Soluble/insoluble ratios commonly change with time and conditions due to complexation, hydrolysis precipitation, agglomeration, or other factors. Particulate size may increase or decrease. Solubles may be sorbed on or desorbed from solid surfaces. Settleability or turbidity are transient phenomena subject to change with time or conditions.

5 Biological progressions are dynamic entities that shift among "critters" in relation to predominence in variety, numbers, growth, and decay. Whatever is, will change in response to nutrition and conditions.

IV SITUATIONS FAVORING FIELD TESTING

A Preliminary information often is needed to guide situation evaluation, problem identification, on site variation with respect to cross section, depth, or time. These data are useful to determine whether there is a problem or not and for planning of subsequent operations.

1 It may be necessary to locate suspected inflows, channels, or sources of items affecting water quality.

2 Definition of mixing zones often is required.

3 Laboratory time may be reduced if approximate concentrations of items sought are known.

4 Field tests or observations may suggest other tests that are more critical in evaluation of the given situation.

5 Stratification may be evaluated for guidance of subsequent operations.

6 Knowledge of the distribution of components is useful for selecting meaningful future sampling sites.

B The field test may be used to check compliance with regulations prior to more rigorous backup testing.

1 An established operation may be in control or out of control.

2 The field test may reveal which of multiple discharges are in conformance.

3 Undisclosed discharges may become apparent.

4 It may be necessary to "track" some hazardous discharge down stream to guide subsequent users on the choice of intake or storage water.

5 A complaint or inquiry may be evaluated by field tests.

C In-plant field type tests are essential to guide operations toward the production of continuous high quality effluents. Record tests commonly are provided too late to do anything about the situation; quick test results are emphasized for process control because time, manpower, and change are critical.

1 Surprises in the form of wastewater changes in flow, concentration, or significant components are common for treatment process operators. They need quick numbers rather than impressions to distinguish real from apparent problems.

2 Process upsets are minimized or prevented by regular and meaningful tests from which trends may be established. Small imbalances may be corrected before they become major upsets.

3 Backup or supplementary treatment such as coagulation, adsorption, neutralization, may prevent serious process disruption if tests indicate the problem and its magnitude in time to do something about it.

V TYPICAL FIELD TESTS - INSTRUMENTAL

A pH is one of the simplest and most valuable field tests. If mineral acids or alkalies in stable form are the contributing factors, then color indicators, comparators, impregnated papers or like devices may be used to obtain an estimate of pH. To obtain a pH value for NPDES report purposes, an electronic sensor (electrode) and extension wire to an indicator instrument (meter) is essential.

This measurement should be done on site because pH is significantly affected by several variables. Temperature directly affects the pH of solutions. Free CO_2 in solution, particularly in samples from the vicinity of benthic deposits or active biological systems, is another major variable. Any delay, temperature or pressure change or sample manipulation will affect the free CO_2 concentration of the solution and thereby alter the pH response.

Electronic pH instruments are produced for field use by many apparatus supply firms. With care, they function effectively.

B Conductivity is a very useful index of ionic materials and usually may be correlated with wet chemical data to give a reasonable correlation with total dissolved solids for a given mixture. This test is very useful to detect saline intrustions or discharges, springs, hidden channels of different salinity from the main body of water. Several reliable instruments are marketed.

C DO analyzers are available for field use application from several manufacturers. They differ in portability, accessory equipment and in versatility so that it is possible to obtain one or more fitting almost any requirement. These units are based upon reduction of oxygen at the cathode surface to convert chemical to electrical energy. Direct measurement of electrical energy produced or the change in some carrier current or voltage may be used for readout. The signal may be amplified in line with sensitivity to give direct concentration readout. Temperature compensation is useful and available. Membrane covered sensors selective for dissolved gases protect sensor surfaces from most interfering components. The analyst must learn to use his particular instrument effectively to enable him to obtain valid results under conditions in which a wet chemical procedure would be misleading.

VI TYPICAL FIELD TESTS - WET CHEMICAL

A DO titrations for clean water samples may be adapted for field use by shifting to dry reagents encased in plastic pillows, substituting phenylarsine oxide for thio and using plastic burets, sample containers, etc. Powdered starch substitutes also are available. Sample sizes may be altered along with reagent concentration to maintain the 1 to 1 ratio of DO to titrant volume. DO test kits are available from several manufacturers. Many agencies make their own.

B Alkalinity and hardness titrations are commonly sought under field conditions using standard acid and EDTA solutions. To obtain alkalinity results for NPDES report purposes, the sample should be titrated to a pH end point using a calibrated pH meter. Powder pillows for buffer and indicator are available to facilitate conducting the hardness titration in the field.

C Chlorine tests using orthotolidine in a comparator frequently are used for rapid on-site measurements. The necessary equipment is readily available from several manufacturers. To obtain results for NPDES report purposes, one must use a titration procedure involving either a starch-iodide, color-change end point or else a meter which provides amperage-change end point detection.

VII SUMMARY

Simple and rapid field test kits are available from several manufacturers, suppliers and scientific apparatus houses for individual tests, as well as combination test kits for the items mentioned and others.

It is possible for the analyst to devise one suited to his particular requirements with available or improvised materials. Effectiveness of the field operation depends on the knowledge and care exercised in using the equipment and procedure.

CAUTION: Field test results are useful, but not always valid enough for the record.

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This outline was prepared by F. J. Ludzack, former Chemist, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Chemical Analysis, On-Site Tests, Water Analysis

BACTERIOLOGICAL INDICATORS OF WATER POLLUTION

Part 1. General Concepts

I INTRODUCTION

A Bacterial Indication of Pollution

1 In the broadest sense, a bacterial indicator of pollution is any organism which, by its presence, would demonstrate that pollution has occurred, and often suggest the source of the pollution.

2 In a more restrictive sense, bacterial indicators of pollution are associated primarily with demonstration of contamination of water, originating from excreta of warm-blooded animals (including man, domestic and wild animals, and birds).

B Implications of Pollution of Intestinal Origin

1 Intestinal wastes from warm-blooded animals regularly include a wide variety of genera and species of bacteria. Among these the coliform group may be listed, and species of the genera Streptococcus, Lactobacillus, Staphylococcus, Proteus, Pseudomonas, certain spore-forming bacteria, and others.

2 In addition, many kinds of pathogenic bacteria and other microorganisms may be released in wastes on an intermittent basis, varying with the geographic area, state of community health, nature and degree of waste treatment, and other factors. These may include the following:

a Bacteria: Species of Salmonella, Shigella, Leptospira, Brucella, Mycobacterium, and Vibrio comma.

b Viruses: A wide variety, including that of infectious hepatitis, Polioviruses, Coxsackie virus, ECHO viruses (enteric cytopathogenic human orphan -- "viruses in search of a disease"), and unspecified viruses postulated to account for outbreaks of diarrheal and upper respiratory diseases of unknown etiology, apparently infective by the water-borne route.

c Protozoa: Endamoeba histolytica

3 As routinely practiced, bacterial evidence of water pollution is a test for the presence and numbers of bacteria in wastes which, by their presence, indicate that intestinal pollution has occurred. In this context, indicator groups discussed in subsequent parts of this outline are as follows:

a Coliform group and certain subgroupings

b Fecal streptococci and certain subgroupings

c Miscellaneous indicators of water quality

4 Evidence of water contamination by intestinal wastes of warm-blooded animals is regarded as evidence of health hazard in the water being tested.

II PROPERTIES OF AN IDEAL INDICATOR OF POLLUTION

A An "ideal" bacterial indicator of pollution should:

1 Be applicable in all types of water

2 Always be present in water when pathogenic bacterial constituents of fecal contamination are present. Ramifications of this include --

  a its density should have some direct relationship to the degree of fecal pollution.

  b It should have greater survival time in water than enteric pathogens, throughout its course of natural disappearance from the water body.

  c It should disappear rapidly from water following the disappearance of pathogens, either through natural or man-made processes.

  d It always should be absent in a bacteriologically safe water.

3 Lend itself to routine quantitative testing procedures without interference or confusion of results due to extraneous bacteria

4 Be harmless to man and other animals

B In all probability, an "ideal" bacterial indicator does not exist. The discussion of bacterial indicators of pollution in the following parts of this outline include consideration of the merits and limitations of each group, with their applications in evaluating bacterial quality of water.

III APPLICATIONS OF TESTS FOR POLLUTION INDICATORS

A Tests for Compliance with Bacterial Water Quality Standards

1 Potability tests on drinking water to meet Interstate Quarantine or other standards of regulatory agencies.

2 Determination of bacterial quality of environmental water for which quality standards may exist, such as shellfish waters, recreational waters, water resources for municipal or other supplies.

3 Tests for compliance with established standards in cases involving the protection or prosecution of municipalities, industries, etc.

B Treatment Plant Process Control

1 Water treatment plants

2 Wastewater treatment plants

C Water Quality and Pollutant Source Monitoring

1 Determination of intestinal pollution in surface water to determine type and extent of treatment required for compliance with standards

2 Tracing sources of pollution

3 Determination of effects on bacterial flora, due to addition of organic or other wastes

D Special Studies, such as

1 Tracing sources of intestinal pathogens in epidemiological investigations

2 Investigations of problems due to the Sphaerotilus group

3 Investigations of bacterial interference to certain industrial processes, with respect to such organisms as Pseudomonas, Achromobacter, or others

IV SANITARY SURVEY

The laboratory bacteriologist is not alone in evaluation of indication of water pollution of intestinal origin. On-site study (Sanitary Survey) of the aquatic environment and adjacent areas, by a qualified person, is a necessary collateral study with the laboratory work and frequently will reveal information regarding potential bacteriological hazard which may or may not be demonstrated through laboratory findings from a single sample or short series of samples.

Part 2. The Coliform Group and Its Constituents

I ORIGINS AND DEFINITION

A Background

1 In 1885, Escherich, a pioneer bacteriologist, recovered certain bacteria from human feces, which he found in such numbers and consistency as to lead him to term these organisms "the characteristic organism of human feces."

He named these organisms *Bacterium coli-commune* and *B. lactis aerogenes*. In 1895, another bacteriologist, Migula, renamed *B. coli commune* as *Escherichia coli*, which today is the official name for the type species.

2 Later work has substantiated much of the original concept of Escherich, but has shown that the above species are in fact a heterogeneous complex of bacterial species and species variants.

a This heterogeneous group occurs not only in human feces but representatives also are to be found in many environmental media, including sewage, surface freshwaters of all categories, in and on soils, vegetation, etc.

b The group may be subdivided into various categories on the basis of numerous biochemical and other differential tests that may be applied.

B Composition of the Coliform Group

1 Current definition

As defined in "Standard Methods for the Examination of Water and Wastewater" (14th ed): "The coliform group includes all of the aerobic and facultative anaerobic, Gram-negative, nonspore-forming rod-shaped bacteria which ferment lactose with gas formation within 48 hours at 35° C."

2 The term "coliforms" or "coliform group" is an inclusive one, including the following bacteria which may meet the definition above:

a *Escherichia coli*, *E. aurescens*, *E. freundii*, *E. intermedia*

b *Enterobacter aerogenes*, *E. cloacae*

c Biochemical intermediates between the genera *Escherichia* and *Enterobacter*

3 There is no provision in the definition of coliform bacteria for "atypical" or "aberrant" coliform strains.

a An individual strain of any of the above species may fail to meet one of the criteria of the coliform group.

b Such an organism, by definition, is not a member of the coliform group, even though a taxonomic bacteriologist may be perfectly correct in classifying the strain in one of the above species.

II SUBDIVISION OF COLIFORMS INTO "FECAL" AND "NONFECAL" CATEGORIES

A Need

Single-test differentiations between coliforms of "fecal" origin and those of "nonfecal" origin are based on the assumption that typical *E. coli* and closely related strains are of fecal origin while *E. aerogenes* and its close relatives are not of direct fecal origin. (The latter assumption is not fully borne out by investigations at this Center. See Table 1, IMViC Type --++). A number of single differential tests have been proposed to differentiate between "fecal" and "nonfecal" coliforms.

Without discussion of their relative merits, several may be cited here:

B Types of Single-Test Differentiation

1 Determination of gas ratio

Fermentation of glucose by E. coli results in gas production, with hydrogen and carbon dioxide being produced in equal amounts. Fermentation of glucose by E. aerogenes results in generation of twice as much carbon dioxide as hydrogen.

Further studies suggested absolute correlation between H_2/CO_2 ratios and the terminal pH resulting from glucose fermentation. This led to the substitution of the methyl red test.

2 Methyl red test

Glucose fermentation by E. coli typically results in a culture medium having terminal pH in the range 4.2 - 4.6 (red color a positive test with the addition of methyl red indicator). E. aerogenes typically results in a culture medium having pH 5.6 or greater (yellow color, a negative test).

3 Indole

When tryptophane, an amino acid, is incorporated in a nutrient broth, typical E. coli strains are capable of producing indole (positive test) among the end products, whereas E. aerogenes does not (negative test).

In reviewing technical literature, the worker should be alert to the method used to detect indole formation, as the results may be greatly influenced by the analytical procedure.

4 Voges-Proskauer test (acetylmethyl carbinol test)

The test is for detection of acetylmethyl carbinol, a derivative of 2, 3, butyleneglycol, as a result of glucose fermentation in the presence of peptone. E. aerogenes produces this end product (positive test) whereas E. coli gives a negative test.

a Experience with coliform cultures giving a positive test has shown a loss of this ability with storage on laboratory media for 6 months to 2½ years, in 20 - 25% of cultures (105 out of 458 cultures).

b Some workers consider that all coliform bacteria produce acetylmethyl carbinol in glucose metabolism. These workers regard acetylmethyl carbinol-negative cultures as those which have enzyme systems capable of further degradation of acetylmethyl carbinol to other end products which do not give a positive test with the analytical procedure. Cultures giving a positive test for acetylmethyl carbinol lack this enzyme system.

c This reasoning leads to a hypothesis (not experimentally proven) that the change of reaction noted in certain cultures in 4.a above is due to the activation of a latent enzyme system.

5 Citrate utilization

Cultures of E. coli are unable to use the carbon of citrates (negative test) in their metabolism, whereas cultures of E. aerogenes are capable of using the carbon of citrates in their metabolism (positive test).

Some workers (using Simmons Citrate Agar) incorporate a pH indicator (brom thymol blue) in the culture medium in order to demonstrate the typical alkaline reaction (pH 8.4 - 9.0) resulting with citrate utilization.

6 Elevated temperature (Eijkman) test

a The test is based on evidence that E. coli and other coliforms of fecal origin are capable of growing and fermenting carbohydrates (glucose or lactose) at temperatures significantly higher than the body temperature of warm-blooded animals. Organisms not associated with direct fecal origin would give a negative test result, through their inability to grow at the elevated temperature.

b While many media and techniques have been proposed, EC Broth, a medium developed by Perry and Hajna, used as a confirmatory medium for 24 hours at 44.5 ± 0.2°C are the current standard medium and method.
While the "EC" terminology of the medium suggests "E. coli" the worker should not regard this as a specific procedure for isolation of E. coli.

c A similar medium, Boric Acid Lactose Broth, has developed by Levine and his associates. This medium gives results virtually identical with those obtained from EC Broth, but requires 48 hours of incubation.

d Elevated temperature tests require incubation in a water bath. Standard Methods 14th Ed. requires this temperature to be 44.5 ± 0.2°C. Various workers have urged use of temperatures ranging between 43.0°C and 46.0°C. Most of these recommendations have provided a tolerance of ± 0.5°C from the recommended levels. However, some workers, notably in the Shellfish Program of the Public Health Service, stipulate a temperature of 44.5 ± 0.2°C. This requires use of a water bath with forced circulation to maintain this close tolerance. This tolerance range was instituted in the 13th Edition of Standard Methods and the laboratory worker should conform to these new limits.

e The reliability of elevated temperature tests is influenced by the time required for the newly-inoculated cultures to reach the designated incubation temperature. Critical workers insist on placement of the cultures in the water bath within 30 minutes, at most, after inoculation.

7 Other tests

Numerous other tests for differentiation between coliforms of fecal vs. nonfecal origin have been proposed. Current studies suggest little promise for the following tests in this application: uric acid test, cellobiose fermentation, gelatin liquefaction, production of hydrogen sulfide, sucrose fermentation, and others.

C IMViC Classification

1 In 1938, Parr reported on a review of a literature survey on biochemical tests used to differentiate between coliforms of fecal vs. nonfecal origin. A summary follows:

Test	No. of times used for differentiation
Voges-Proskauer reaction	22
Methyl red test	20
Citrate utilization	20
Indole test	15
Uric acid test	6
Cellobiose fermentation	4
Gelatin liquefaction	3
Eijkman test	2
Hydrogen sulfide production	1
Sucrose fermentation	1
a-Methyl-d-glucoside fermentation	1

2 Based on this summary and on his own studies, Parr recommended utilization of a combination of tests, the indole, methyl red, Voges-Proskauer, and the citrate utilization tests for this differentiation. This series of reactions is designated by the mnemonic "IMViC". Using this scheme, any coliform culture can be described by an "IMViC Code" according to the reactions for each culture. Thus, a typical culture of *E. coli* would have a code ++--, and a typical *E. aerogenes* culture would have a code --++.

3 Groupings of coliforms into fecal, non-fecal, and intermediate groups, as shown in "Standard Methods for the Examination of Water and Wastewater" are shown at the bottom of this page.

D Need for Study of Multiple Cultures

All the systems used for differentiation between coliforms of fecal vs. those of nonfecal origin require isolation and study of numerous pure cultures. Many workers prefer to study at least 100 cultures from any environmental source before attempting to categorize the probable source of the coliforms.

III NATURAL DISTRIBUTION OF COLIFORM BACTERIA

A Sources of Background Information

Details of the voluminous background of technical information on coliform bacteria recovered from one or more environmental media are beyond the scope of this discussion. References of this outline are suggested routes of entry for workers seeking to explore this topic.

B Studies on Coliform Distribution

1 Since 1960 numerous workers have engaged in a continuing study of the natural distribution of coliform bacteria and an evaluation of procedures for differentiation between coliforms of fecal vs. probable nonfecal origin. Results of this work have special significance because:

a Rigid uniformity of laboratory methods have been applied throughout the series of studies

b Studies are based on massive numbers of cultures, far beyond any similar studies heretofore reported

INTERPRETATION OF IMViC REACTIONS

Organism	Indole	Methyl Red	Voges-Proskauer	Citrate
Escherichia coli	+ or -	+	-	-
Citrobacter freundii	-	+	-	+
Klebsiella-Enterobacter group	+ or -	-	+	+

c A wider variety of environmental and biological sources are being studied than in any previous series of reports.

d All studies are based on freshly recovered pure culture isolates from the designated sources.

e All studies are based on cultures recovered from the widest feasible geographic range, collected at all seasons of the year.

2 Distribution of coliform types

Table 1 shows the consolidated results of coliform distributions from various biological and environmental sources.

a The results of these studies show a high order of correlation between known or probable fecal origin and the typical E. coli IMViC code (++--). On the other hand, human faces also includes numbers of E. aerogenes and other IMViC types, which some regard as "nonfecal" segments of the coliform group. (Figure 1)

b The majority of coliforms attributable to excretal origin tend to be limited to a relatively small number of the possible IMViC codes; on the other hand, coliform bacteria recovered from undisturbed soil, vegetation, and insect life represent a wider range of IMViC codes than fecal sources, without clear dominance of any one type. (Figure 2)

c The most prominant IMViC code from nonfecal sources is the intermediate type, -+-+, which accounts for almost half the coliform cultures recovered from soils, and a high percentage of those recovered from vegetation and from insects. It would appear that if any coliform segment could be termed a "soil type" it would be IMViC code -+-+.

d It should not be surprising that cultures of typical E. coli are recovered in relatively smaller numbers from sources judged, on the basis of sanitary survey, to be unpolluted. There is no known way to exclude the influence of limited fecal pollution from small animals and birds in such environments.

e The distribution of coliform types from human sources should be regarded as a representative value for large numbers of sources. Investigations have shown that there can be large differences in the distribution of IMViC types from person to person, or even from an individual.

3 Differentiation between coliforms of fecal vs. nonfecal origin

Table 2 is a summary of findings based on a number of different criteria for differentiating between coliforms of fecal origin and those from other sources.

a IMViC type ++-- is a measurement of E. coli, Variety I, and appears to give reasonably good correlation between known or highly probable fecal origin and doubtful fecal origin.

b The combination of IMViC types, ++--, +---, and -+--, gives improved identification of probable fecal origin, and appears also to exclude most of the coliforms not found in excreta of warm-blooded animals in large numbers.

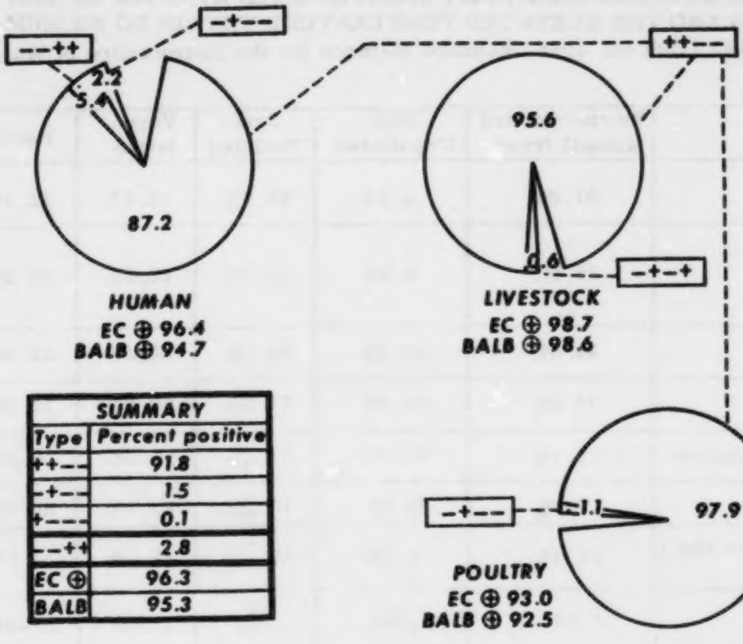
c While the indole, methyl red, Voges Proskauer, and citrate utilization tests, each used alone, appear to give useful answers when applied only to samples of known pollution from fecal sources, the interpretation is not as clear when applied to coliforms from sources believed to be remote from direct fecal pollution.

Table 1. COLIFORM DISTRIBUTION BY IMViC TYPES AND ELEVATED TEMPERATURE TEST FROM ENVIRONMENTAL AND BIOLOGICAL SOURCES

IMViC type	Vegetation		Insects		Soil				Fecal sources				Poultry	
					Undisturbed		Polluted		Human		Livestock			
	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total
++--	128	10.6	134	12.4	131	5.6	536	80.6	3932	87.2	2237	95.6	1857	97.9
--++	237	19.7	113	10.4	443	18.8	13	2.0	245	5.4	0	<0.1	1	0.1
-+--	23	1.9	0	<0.1	78	3.3	1	0.2	99	2.2	14	0.6	20	1.1
+++-	2	0.2	0	<0.1	7	0.3	0	<0.1	106	2.4	59	2.5	0	<0.1
-+-+	168	14.0	332	30.6	1131	48.1	87	13.0	50	1.1	1	<0.1	5	0.3
++-+	116	9.6	118	10.9	87	3.7	22	3.3	35	0.8	27	1.2	11	0.6
-+++	32	2.7	28	2.6	181	7.7	5	0.7	21	0.5	0	<0.1	0	<0.1
++++	291	24.2	254	23.4	159	6.8	0	<0.1	6	0.1	0	<0.1	0	<0.1
+-++	88	7.3	46	4.2	67	2.9	0	<0.1	14	0.2	0	<0.1	0	<0.1
---+	87	7.2	42	3.9	4	0.2	1	0.2	2	<0.1	0	<0.1	0	<0.1
-++-	5	0.4	0	<0.1	1	<0.1	0	<0.1	0	<0.1	0	<0.1	0	<0.1
--+-	19	1.6	0	<0.1	53	2.3	0	<0.1	0	<0.1	0	<0.1	0	<0.1
+-+-	2	0.2	0	<0.1	6	0.3	0	<0.1	0	<0.1	0	<0.1	0	<0.1
+--+	5	0.4	8	0.7	0	<0.1	0	<0.1	0	<0.1	0	<0.1	0	<0.1
+---	0	<0.1	9	0.8	0	<0.1	0	<0.1	2	<0.1	0	<0.1	2	<0.1
Total	1203		1084		2348		665		4512		2339		1896	
No. EC +	169*		162*		216		551		4349		2309		1765	
% EC +	14.1*		14.9*		9.2		82.9		96.4		98.7		93.0	

*120 of these were ++--, 15 --++, 11 -+--

*129 of these were ++--, 27 -+-+, 5 ++-+



SUMMARY	
Type	Percent positive
++--	91.8
-+--	1.5
+---	0.1
--++	2.8
EC ⊕	96.3
BALB	95.3

FIGURE 1

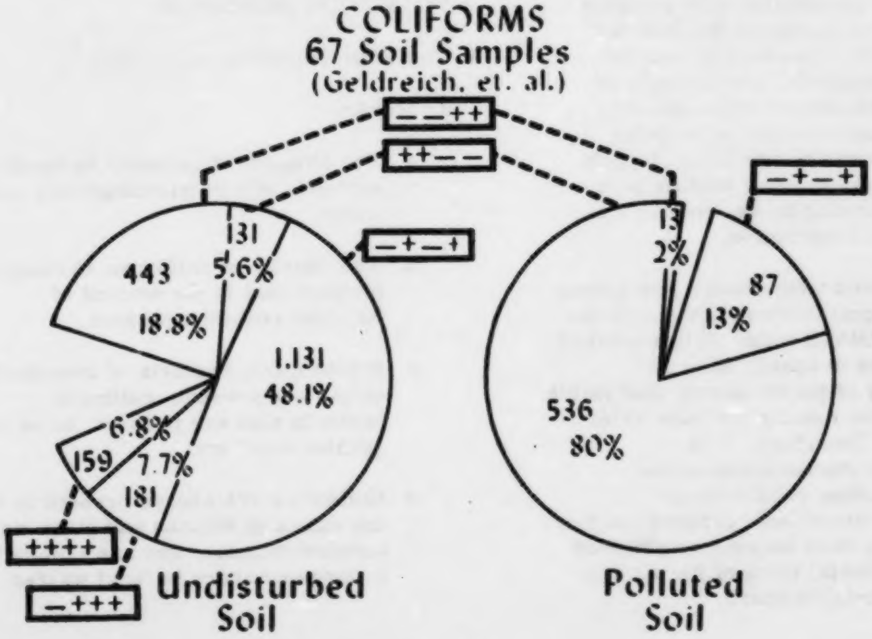


FIGURE 2

Table 2. COMPARISON OF COLIFORM STRAINS ISOLATED FROM WARM-BLOODED ANIMAL FECES, FROM UNPOLLUTED SOILS AND POLLUTED SOILS WITH USE OF THE IMViC REACTIONS AND THE ELEVATED TEMPERATURE TEST IN EC MEDIUM AT 44.5° C (±0.5°) (12th ed. 1965; Standard Methods for the Examination of Water and Wastewater)

Test	Warm-blooded animal feces	Soil: Unpolluted	Soil: Polluted	Vegetation	Insects
+ + - -	91.8%	5.6%	80.6%	10.6%	12.4%
+ + - -, + - - - and - + - -	93.3%	8.9%	80.7%	12.5%	13.2%
Indole positive	94.0%	19.4%	82.7%	52.5%	52.4%
Methyl red positive	96.9%	75.6%	97.9%	63.6%	79.9%
Voges-Proskauer positive	5.1%	40.7%	97.3%	56.3%	40.6%
Citrate utilizers	3.6%	88.2%	19.2%	85.1%	86.7%
Elevated temperature (EC) positive	96.4%	9.2%	82.9%	14.1%	14.9%
Number of cultures studied	8,747	2,348	665	1,203	1,084

Total Pure Cultures Studied: 14,047

d The elevated temperature test gives excellent correlation with samples of known or highly probable fecal origin. The presence of smaller, but demonstrable, percentages of such organisms in environmental sources not interpreted as being polluted could be attributed largely to the warm-blooded wildlife in the area, including birds, rodents, and other small mammals.

e The elevated temperature test yields results equal to those obtained from the total IMViC code. It has marked advantages in speed, ease and simplicity of performance, and yields quantitative results for each water sample. Therefore, it is the official standard method for differentiation coliforms of probable direct fecal origin from those which may have become established in the bacterial flora of the aquatic or terrestrial habitat.

IV EVALUATION OF COLIFORMS AS POLLUTION INDICATORS

A The Coliform Group as a Whole

1 Merits

a The absence of coliform bacteria is evidence of a bacteriologically safe water.

b The density of coliforms is roughly proportional to the amount of excretal pollution present.

c If pathogenic bacteria of intestinal origin are present, coliform bacteria also are present, in much greater numbers.

d Coliforms are always present in the intestines of humans and other warm-blooded animals, and are eliminated in large numbers in fecal wastes.

e Coliforms are more persistent in the aquatic environment than are pathogenic bacteria of intestinal origin.

f Coliforms are generally harmless to humans and can be determined quantitatively by routine laboratory procedures.

2 Limitations

a Some of the constituents of the coliform group have a wide environmental distribution in addition to their occurrence in the intestines of warm-blooded animals.

b Some strains of the coliform group may multiply in certain polluted waters ("aftergrowth"), of high nutritive values thereby adding to the difficulty of evaluating a pollution situation in the aquatic environment. Members of the E. aerogenes section of the coliform are commonly involved in this kind of problem.

c Because of occasional aftergrowth problems, the age of the pollution may be difficult to evaluate under some circumstances.

d Tests for coliforms are subject to interferences due to other kinds of bacteria. False negative results sometimes occur when species of Pseudomonas are present. False positive results sometimes occur when two or more kinds of non-coliforms produce gas from lactose, when neither can do so alone (synergism).

B The Fecal Coliform Component of the Coliform Group (as determined by elevated temperature test)

1 Merits

a The majority (over 95% of the coliform bacteria from intestines of warm-blooded animals grow at the elevated temperature.

b These organisms are of relatively infrequent occurrence except in association with fecal pollution.

c Survival of the fecal coliform group is shorter in environmental waters than for the coliform group as whole. It follows, then, that high densities of fecal coliforms is indicative of relatively recent pollution.

d Fecal coliforms generally do not multiply outside the intestines of warm-blooded animals. In certain high-carbohydrate wastes, such as from the sugar beet refineries, exceptions have been noted.

e In some wastes, notably those from pulp and paper mills, Klebsiella has been found in large numbers utilizing the elevated temperature test. There has been much controversy about whether the occurrence of Klebsiella is due to aftergrowth due to soluble carbohydrates in such wastes. The significance of Klebsiella as an indicator of direct discharge of intestinal wastes thus is under challenge. The issue is still further complicated by questions over whether Klebsiella is in and of itself a pathogenic organism or is potentially pathogenic. This is a serious problem which is the subject of intensive research efforts.

2 Limitations

a Feces from warm-blooded animals include some (though proportionately low) numbers of coliforms which do not yield a positive fecal coliform test when the elevated temperature test is used as the criterion of differentiation. These organisms are E. coli varieties by present taxonomic classification.

b There is at present no established and consistent correlation between ratios of total coliforms/fecal coliforms in interpreting sanitary quality of environmental waters.

In domestic sewage, the fecal coliform density commonly is greater than 90% of the total coliform density. In environmental waters relatively free from recent pollution, the fecal coliform density may range from 10-30% of the total coliforms. There are, however, too many variables relating to water-borne wastes and surface water runoff to permit sweeping generalization on the numerical relationships between fecal- and total coliforms.

c Studies have been made regarding the survival of fecal coliforms in polluted waters compared with that of enteric pathogenic bacteria. In recent pollution studies, species of Salmonella have been found in the presence of 220 fecal coliforms per 100 ml (Spino), and 110 fecal coliforms per 100 ml (Brezenski, Raritan Bay Project).

3 The issue of the Klebsiella problem described in an earlier paragraph may ultimately be resolved as a merit or as a limitation of the value of the fecal coliform test.

V APPLICATIONS OF COLIFORM TESTS

A Current Status in Official Tests

1 The coliform group is designated, in "Standard Methods for the Examination of Water and Wastewater" (14th ed., 1975), through the Completed Test MPN procedure as the official test for bacteriological potability of water. The Confirmed Test MPN procedure is accepted where it has been demonstrated, through comparative tests, to yield results equivalent to the Completed Test. The membrane filter method also is accepted for examination of waters subject to interstate regulation.

2 The 12th edition of Standard Methods introduced a standard test for fecal coliform bacteria. It is emphasized that this is to be used in pollution studies, and does not apply to the evaluation of water for potability. This procedure has been continued in the 13th and 14th Editions.

B Applications

1 Tests for the coliform group as a whole are used in official tests to comply with interstate drinking water standards, state standards for shellfish waters, and in most, if not all, cases where bacterial standards of water quality have been established for such use as in recreational or bathing waters, water supplies, or industrial supplies. Laboratory personnel should be aware of possible implementation of the fecal coliform group as the official test for recreational and bathing waters.

2 The fecal coliform test has application in water quality surveys, as an adjunct to determination of total coliform density. The fecal coliform test is being used increasingly in all water quality surveys.

3 It is emphasized that no responsible worker advocates substitution of a fecal coliform test for total coliforms in evaluating drinking water quality.

Part 3. The Fecal Streptococci

I INTRODUCTION

Investigations regarding streptococci progressed from the streptococci of medical concern to those which were distributed in differing environmental conditions which, again, related to the welfare of man. The streptococci were originally reported by Laws and Andrews (1894), and Houston (1899, 1900) considered those streptococci, which we now call "fecal streptococci," as ... "indicative of dangerous pollution, since they are readily demonstrable in waters recently polluted and seemingly altogether absent from waters above suspicion of contamination.

From their discovery to the present time the fecal streptococci appear characteristic of fecal pollution, being consistently present in both the feces of all warm-blooded animals and in the environment associated with animal discharges. As early as 1910 fecal streptococci were proposed as indicators to the Metropolitan Water Board of London. However, little progress resulted in the United States until improved methods of detection and enumeration appeared after World War II.

Renewed interest in the group as indicators began with the introduction of azide dextrose broth in 1950, (Mallmann & Seligmann, 1950). The method which is in the current edition of Standard Methods appeared soon after. (Litsky, et al. 1955).

With the advent of improved methods for detection and enumeration of fecal streptococci, significant body of technical literature has appeared.

This outline will consider the findings of various investigators regarding the fecal streptococci and the significance of discharges of these organisms into the aquatic environment.

II FECAL MATERIALS

A Definition

The terms "enterococci," "fecal streptococci," "Group D streptococci," "Streptococcus fecalis," and even "streptococci" have been used in a loose and interchangeable manner to indicate the streptococci present in the enteric tract of warm-blooded animals or of the fresh fecal material excreted therefrom.

Enterococci are characterized by specific taxonomic biochemistry. Serological procedures differentiate the Group D streptococci from the various groups. Although they overlap, the three groups, fecal streptococcus, enterococcus, and Group D streptococcus, are not synonymous. Because our emphasis is on indicators of unsanitary origin, fecal streptococcus is the more appropriate term and will include the enterococcus as well as other groups.

Increasing attention is being paid to certain streptococci found in humans and certain birds whic were, at one time, considered to be biotypes of Str. faecalis or Str. faecium and therefore legitimate fecal streptococci. These are now considered to be in a separate group in their own right, the Group Q streptococci.

A rigid definition of the fecal streptococcus group is not possible with our present knowledge. The British Ministry of Health (1956) defines the organisms as "Gram-positive" cocci, generally occurring in pairs or short chains, growing in the presence of bile salt, usually capable of development at 45° C, producing acid but not gas in mannitol and lactose, failing to attack raffinose, failing to reduce nitrate to nitrite, producing acid in litmus milk and precipitating the casein in the form of a loose, but solid curd, and exhibiting a greater resistance to heat, to alkaline conditions and to high concentrations of salt than most vegetative bacteria." However, it is pointed out that "streptococci departing in one or more particulars from the type species cannot be disregarded in water."

Standard Methods (14th ed., 1975) describes the fecal streptococci as pure culture selective medium organisms which are Catalase negative and capable of originating growth in BHI broth (45°C. for 48 hours) and Bile broth medium (35° C. for 3 days).

For the proposes of this outline, and in line with the consensus of most water microbiologists in this country, the general definition of the fecal streptococci is:

> . . . "The group composed of Group D and Q species consistently present in significant numbers in fresh fecal excreta of warm-blooded animals, which includes all of the enterococcus group in addition to other groups of streptococci."

B Species Isolated

1 Findings

a Human feces

Examination of human fecal specimens yields a high percentage of the enterococcus group and usually demonstration of the S. salivarius which is generally considered a member of the human throat flora and to be surviving in human fecal materials rather than actively multiplying in the enteric tract. Also present would be a small percentage of variants or biotypes of the enterococcus group.

b Nonhuman Feces

1) Fecal material which are from nonhuman and not from fowl will yield high percentages of the S. bovis and/or S. equinus organisms with a concomitantly reduced percentage of the enterococcus group.

2) Fowl excreta

Excrement from fowl characteristically yields a large percentage of enterococcal biotypes (Group Q) as well as a significant percentage of enterococcus group.

2 Significance

Species associations with particular animal hosts is an established fact and leads to the important laboratory technique of partition counting of colonies from the membrane filter or agar pour plates in order to establish or confirm the source of excretal pollution in certain aquatic investigations.

It is important to realize that a suitable medium is necessary in order to allow all of the streptococci which we consider to be fecal streptococci to grow in order to give credence to the derived opinions. Use of liquid growth media into which direct inoculations from the sample are made have not proven to be successful for partition counting due to the differing growth rates of the various species of streptococci altering the original percentage relationships. Due to the limited survival capabilities of some of the fecal streptococci it is necessary to sample fresh fecal material or water samples in close proximity to the pollution source especially when multiple sources are contributing to a reach of water. Also the pH range must be within the range of 4.0-9.0.

Standard Methods (14th ed., 1975) now includes a schematic allowing for the identification of fecal streptococci types present within a given sample.

III FECAL STREPTOCOCCI IN THE AQUATIC ENVIRONMENT

A General

From the foregoing it is appears that the preponderant human fecal streptococci are composed of the enterococcus group and, as this is the case, several media are presently available which will detect only the enterococcal group will be suitable for use with aquatic samples which are known to be contaminated or potentially contaminated with purely domestic (human) wastes. On the other hand, when it is known or suspected that other-than-human wastes have potential egress to the aquatic environment under investigation, it is necessary to utilize those media which are capable of quantitating the whole of the fecal streptococci group.

B Stormwaters and Combined Sewers

1 General

Storm sewers are a series of pipes and conduits which receive surface runoffs from the action of rainstorms and do not include sewage which are borne by a system of sanitary sewers. Combined sewers receive both the storm runoff and the water-borne wastes of the sanitary system. Both storm water and combined sewer flows have been found to usually contain large quantities of fecal streptococci in numbers which generally are larger than those of the fecal coliform indicator organisms.

2 Bacteriological Findings

Table 1 represents, in a modified form, some of the findings of Geldreich and Kenner (1969) with respect to the densities of fecal streptococci when considering Domestic sewage in contrast to Stormwaters:

The Ratio FC/FS is that of the Fecal coliform and Fecal streptococci and it will be noted that in each case, when considering the Domestic Sewage, it is 4.0 or greater while it is less than 0.7 for stormwaters. The use of this ratio is useful to identify the source of pollution as being human or nonhuman warm-blooded animal polluted. When the ratio is greater than 4.0 it is considered to be human waste contaminated while a ratio of less than 0.7 is considered to be nonhuman. It is evident that the stormwaters have been primarily polluted by excreta of rats and other rodents and possibly domestic and/or farm animals.

Species differences are the main cause of different fecal coliform-fecal streptococci ratios. Table 2 compares fecal streptococcus and fecal coliform counts for different species. Even though individuals vary widely, masses of individuals in a species have characteristic proportion of indicators.

C Surface Waters

In general, the occurrence of fecal streptococci indicates fecal pollution and its absence indicates that little or no warm-blooded fecal contribution. In studies of remote surface waters the fecal streptococci are infrequently isolated and occurrences of small numbers can be attributed to wild life and/or snow melts and resultant drainage flows.

Various examples of fecal streptococcal occurrences are shown in Table 3 in relation to surface waters of widely varying quality. (Geldreich and Kenner 1969)

IV FECAL STREPTOCOCCI: ADVANTAGES AND LIMITATIONS

A General

Serious studies concerning the streptococci were instituted when it became apparent that they were the agents responsible or suspected for a wide variety of human diseases. Natural priority then focused itself to the taxonomy of these organisms and this study is still causing consternation as more and more microbiological techniques have been brought to bear on these questions. The sanitary microbiologist is concerned with those streptococci which inhabit the enteric tract of warm-blooded animals, their detection, and utilization in developing a criterium for water quality standards.

Table 1

DISTRIBUTION OF FECAL STREPTOCOCCI IN DOMESTIC SEWAGES AND STORMWATER RUNOFFS

Water Source	Fecal Streptococci per 100 ml median values	Ratio FC/FS
Domestic Sewage		
Preston, ID	64,000	5.3
Fargo, ND	290,000	4.5
Moorehead, MN	330,000	4.9
Cincinnati, OH	2,470,000	4.4
Lawrence, MA	4,500,000	4.0
Monroe, MI	700,000	27.9
Denver, CO	2,900,000	16.9
Stormwater		
Business District	51,000	0.26
Residential	150,000	0.04
Rural	58,000	0.05

Table 3

INDICATOR ORGANISMS IN SURFACE WATERS

Water Source	Densities/100 ml	
	Fecal coliform	Fecal streptococci
Prairie Watersheds		
Cherry Creek, WY	90	83
Saline River, KS	95	180
Cub River, ID	110	160
Clear Creek, CO	170	110
Recreational Waters		
Lake Mead	2	444
Lake Moovalaya	9	170
Colorado River	4	256
Whitman River	32	88
Merrimack River	100	96
Public Water Intakes		
Missouri River (1959)		
Mile 470.5	11,500	39,500
Mile 434.5	22,000	79,000
Mile 408.8	14,000	59,000

Table 2. ESTIMATED PER CAPITA CONTRIBUTION OF INDICATOR MICROORGANISMS FROM SOME ANIMALS*

		Average indicator density per gram of feces		Average contribution per capita per 24 hr		
Animals	Avg wt of Feces/24 hr, wet wt, g	Fecal coliform, million	Fecal streptococci, million	Fecal coliform, million	Fecal streptococci, million	Ratio FC/FS
Man	150	13.0	3.0	2,000	450	4.4
Duck	336	33.0	54.0	11,000	18,000	0.6
Sheep	1,130	16.0	38.0	18,000	43,000	0.4
Chicken	182	1.3	3.4	240	620	0.4
Cow	23,600	0.23	1.3	5,400	31,000	0.2
Turkey	448	0.29	2.8	130	1,300	0.1
Pig	2,700	3.3	84.0	8,900	230,000	0.04

*Publication WP-20-3, P. 102

Kabler (1962) discussed the slow acceptance of the fecal streptococci as indicators of pollution resulting from:

1 Multiplicity and difficulty of laboratory procedures

2 Poor agreement between methods of quantitative enumeration

3 Lack of systematic studies of . . . .

a sources

b survival, and

c interpretations, and

4 Undue attention to the S. faecalis group.

Increased attention to the fecal streptococci, especially during the last decade, have clarified many of the earlier cloudy issues and have elevated the stature of these organisms as indicators of pollution. Court precedents establishing legal status and recommendations of various technical advisory boards have placed the fecal coliform group in a position of primacy in many water quality applications. The fecal streptococci have evolved from a position of a theoretically useful indicator to one which was ancillary to the coliforms to one which was useful when discrepancies or questions evolved as to the validity of the coliform data to one where an equality status was achieved in certain applications. In the future it is anticipated that, for certain applications, the fecal streptococci will achieve a position of primacy for useful data, and, as indicated by Litsky (1955) "be taken out of the realm of step-children and given their legitimate place in the field of santiary bacteriology as indicators of sewage pollution."

B Advantages and Limitations

1 Survival

In general, the fecal streptococci have been observed to have a more limited survival time in the aquatic environment when compared to the coliform group. They are rivaled in this respect only by the fecal coliforms. Except for cases of persistence in waters of high electrolytic content, as may be common to irrigation waters, the fecal streptococci have not been observed to multiply in polluted waters as may sometimes be observed for some of the coliforms. Fecal streptococci usually require a greater abundance of nutrients for survival as compared to the coliforms and the coliforms are more dependent upon the oxygen tension in the waterbody. In a number of situations it was concluded that the fecal streptococci reached an extinction point more rapidly in warmer waters while the reverse was true in the colder situations as the coliforms now were totally eliminated sooner.

2 Resistance to Disinfection

In artificial pools the source of contamination by the bathers is usually limited to throat and skin flora and thus increasing attention has been paid to indicators other than those traditionally from the enteric tract. Thus, one of the organisms considered to be a fecal streptococci, namely, S. salivarius, can be a more reliable indicator when detected along with the other fecal streptococci especially since studies have confirmed the greater resistance of the fecal streptococci to chlorination. This greater resistance to chlorination, when compared to the fecal coliforms, is important since the dieoff curve differences are insignificant when the curves of the fecal coliforms are compared to various Gram negative pathogenic bacteria which reduces their effectiveness as indicators.

3 Ubiquitous Strains

Among the fecal streptococcus are two organisms, one a biotype and the other a variety of the S. faecalis, which, being ubiquitous (omnipresent) have limited sanitary significance.

The biotype, or atypical, S. faecalis is characterized by its ability to hydrolyze starch while the varietal form, liquefaciens, is nonbeta haemolytic and capable of liquefying gelatin. Quantitation of these organisms in anomalous conditions is due to their capability of survival in soil or high electrolytic waters and in waters with a temperature of less than 12 Degrees C.

Samples have been encountered which have been devoid of fecal coliforms and yet contain a substantial number of "fecal streptococci" of which these ubiquitous strains constitute the majority or all of the isolations when analyzed biochemically.

V STANDARDS AND CRITERIA

Acceptance and utilization of Total Coliform criteria, which must now be considered a pioneering effort, has largely been supplanted in concept and in fact by the fecal coliforms in establishing standards for recreational waters.

The first significant approach to the utilization of the fecal streptococci as a criterium for recreational water standards occurred in 1966 when a technical committee recommended the utilization of the fecal streptococci with the total coliforms as criteria for standards pertaining to the Calumet River and lower Lake Michigan waters. Several sets of criteria were established to fit the intended uses for this area. The use of the fecal streptococci as a criterium is indicated to be tentative pending the accumulation of existing densities and could be modified in future standards.

With the existing state-of-the-art knowledge of the presence of the fecal streptococci in waters containing low numbers of fecal coliforms it is difficult to establish a specific fecal streptococcus density limit of below 100 organisms/100 ml when used alone or in conjunction with the total coliforms.

The most useful application of the fecal streptococcus test is in the development of the fecal coliform: fecal streptococcus ratio as previously described.

Part 4. Other Bacterial Indicators of Pollution

I TOTAL BACTERIAL COUNTS

A Historical

1 The early studies of Robert Koch led him to develop tentative standards of water quality based on a limitation of not more than 100 bacterial colonies per ml on a gelatin plating medium incubated 3 days at 20° C.

2 Later developments led to inoculation of samples on duplicate plating media, with one set incubated at 37° C and the other at 20° C.

  a Results were used to develop a ratio between the 37° C counts and the 20° C counts.

  b Waters having a predominant count at 37° C were regarded as being of probable sanitary significance, while those giving predominant counts at 20° C were considered to be of probable soil origin, or natural inhabitants of the water being examined.

B Groups Tested

There is no such thing as "total" bacterial count in terms of a laboratory determination.

1 Direct microscopic counts do not differentiate between living and dead cells.

2 Plate counting methods enumerate only the bacteria which are capable of using the culture medium provided, under the temperature and other growth conditions used as a standard procedure. No one culture medium and set of growth conditions can provide, simultaneously, an acceptable environment for all the heterogeneous, often conflicting, requirements of the total range of bacteria which may be recovered from waters.

C Utilization of Total Counts

1 Total bacterial counts, using plating methods, are useful for:

  a Detection of changes in the bacterial composition of a water source

  b Process control procedures in treatment plant operations

  c Determination of sanitary conditions in plant equipment or distributional systems

2 Serious limitations in total bacterial counts exist because:

  a No information is given regarding possible or probable fecal origin of bacterial changes. Large numbers of bacteria can sometimes be cultivated from waters known to be free of fecal pollution.

  b No information of any kind is given about the species of bacteria cultivated.

  c There is no differentiation between harmless or potentially dangerous forms.

3 Status of total counts

Methodology for the determination of the Standard Plate Count has been retained in the 14th Edition of Standard Methods for the stated reason:

> ... "total counts may yield useful information about the quality of water and supporting data on the significance of coliform results ... also, useful in judging the efficiency in operation of various water treatment processes and may have significant application as an in-plant control test. It is also valuable for periodic checking of finished distribution water"
> (abridged for this inclusion)

Technique for the Standard Plate Count is necessary for the performance of the Distilled Water Suitability Test as outlined in Standard Methods and elsewhere within this manual.

B Spore-Forming Bacteria (Clostridium perfringens, or C. welchii)

1 Distribution

This is one of the most widely distributed species of bacteria. It is regularly present in the intestinal tract of warm-blooded animals.

2 Nature of organism

C. perfringens is a Gram-positive, spore-forming rod. The spores cause a distinct swelling of the cell when formed. The organism is extremely active in fermentation of carbohydrates, and produces the well-known "stormy fermentation" of milk.

3 Status

The organism, when present, indicates that pollution has occurred at some time. However, because of the extremely extended viability of the spores, it is impossible to obtain even an approximation of the recency of pollution based only on the presence of C. perfringens.

The presence of the organism does not necessarily indicate an unsafe water.

C Tests for Pathogenic Bacteria of Intestinal Origin

1 Groups considered include Salmonella sp, Shigella sp, Vibrio comma, Mycobacterium sp, Pasteurella sp, Leptospira sp, and others.

2 Merits of direct tests:

Demonstration of any pathogenic species would demonstrate an unsatisfactory water quality, hazardous to persons consuming or coming into contact with that water.

3 Limitations

a There is no available routine procedure for detection of the full range of pathogenic bacteria cited above.

b Quantitative methods are not available for routine application to any of the above.

c The intermittent release of these pathogens makes it impossible to regard water as safe, even in the absence of pathogens.

d After detection, the public already would have been exposed to the organism; thus, there is no built-in margin of safety, as exists with tests for the coliform group.

4 Applications

a In tracing the source of pathogenic bacteria in epidemiological investigations

b In special research projects

c In water quality studies concerned with enforcement actions against pollution, increasing attention is being given to the demonstration of enteric pathogenic bacteria in the presence of the bacterial indicators of pollution.

D Miscellaneous Indicators

It is beyond this discussion to explore the total range of microbiological indicators of pollution that have been proposed and investigated to some extent. Mention can be made, however, of consideration of tests for the following.

1 Bacteriophages specific for any of a number of kinds of bacteria

2 Tests for Enterovirus

3 Serological procedures for detection of coliforms and other indicators: a certain amount of recent attention has been given to applications of fluorescent antibodies in such tests

4 Tests for Klebsiella

5 Tests for *Pseudomonas aeruginosa*

6 Tests for Salmonella

7 Tests for Fungi

8 Tests for Staphylococcus

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This outline was prepared by H. L. Jeter, Former Chief, Program Support Training Branch, National Training and Operational Technology Center, and revised by R. Russomanno, Microbiologist, USEPA, Cincinnati, Ohio 45268

Descriptors: Coliforms, Escherichia coli, Fecal Coliforms, Fecal Streptococci, Indicator Bacteria, Microbiology, Sewage Bacteria, Water Pollution

EXAMINATION OF WATER FOR COLIFORM AND FECAL STREPTOCOCCUS GROUPS
(Multiple Dilution Tube [MPN] Methods)

I INTRODUCTION

The subject matter of this outline is contained in three parts, as follows:

A Part 1

1 Fundamental aspects of multiple dilution tube ("most probable numbers") tests, both from a qualitative and a quantitative viewpoint.

2 Laboratory bench records.

3 Useful techniques in multiple dilution tube methods.

4 Standard supplies, equipment, and media in multiple dilution tube tests.

B Part 2

Detailed, day-by-day, procedures in tests for the coliform group and subgroups within the coliform group.

C Part 3

Detailed, day-by-day, procedures in tests for members of the fecal streptococci.

D Application of Tests to Routine Examinations

The following considerations (Table 1) apply to the selection of the Presumptive Test, the Confirmed Test, and the Completed Test. Termination of testing at the Presumptive Test level is not practiced by laboratories of this agency. It must be realized that the Presumptive Test alone has limited use when water quality is to be determined.

TABLE 1

	Examination Terminated at -		
Type of Receiving Water	Presumptive Test	Confirmed Test	Completed Test
Sewage Receiving	Applicable	Applicable	Important where results are to be used for control of raw or finished water. Application to a statistically valid number of samples from the Confirmed Test to establish its validity in determining the sanitary quality.
Treatment Plant - Raw	Applicable	Applicable	
Chlorinated	Not Done	Applicable	
Bathing	Not Done	Applicable	
Drinking	Not Done	Applicable	
Other Information		Applicable in all cases where Presumptive Test alone is unreliable.	

NOTE: Mention of commercial products and manufacturers does not imply endorsement by the Environmental Protection Agency.

II BASIS OF MULTIPLE TUBE TESTS

A Qualitative Aspects

1 For purely qualitative aspects of testing for indicator organisms, it is convenient to consider the tests applied to one sample portion, inoculated into a tube of culture medium, and the follow-up examinations and tests on results of the original inoculation. Results of testing procedures are definite: *positive* (presence of the organism-group is demonstrated) or *negative* (presence of the organism-group is not demonstrated.)

2 Test procedures are based on certain fundamental assumptions:

- a First, even if only one living cell of the test organism is present in the sample, it will be able to grow when introduced into the primary inoculation medium;
- b Second, growth of the test organism in the culture medium will produce a result which indicates presence of the test organism; and,
- c Third, extraneous organisms will not grow, or if they do grow, they will not limit growth of the test organism; nor will they produce growth effects that will be confused with those of the bacterial group for which the test is designed.

3 Meeting these assumptions usually makes it necessary to conduct the tests in a series of stages (for example, the Presumptive, Confirmed, and Completed Test stages, respectively, of standard tests for the coliform group).

4 Features of a full, multi-stage test

- a *First stage*: The culture medium usually serves primarily as an enrichment medium for the group tested. A good first-stage growth medium should support growth of *all* the living cells of the group tested, and it should include provision for indicating the presence of the test organism being studied. A first-stage medium may include some component which inhibits growth of extraneous bacteria, but this feature *never* should be included if it also inhibits growth of any cells of the group for which the test is designed. The Presumptive Test for the coliform group is a good example. The medium supports growth, presumably, of all living cells of the coliform group; the culture container has a fermentation vial for demonstration of gas production resulting from lactose fermentation by coliform bacteria, if present; and sodium lauryl sulfate may be included in one of the approved media for suppression of growth of certain noncoliform bacteria. This additive apparently has no adverse effect on growth of members of the coliform group in the concentration used. If the result of the first-stage test is negative, the study of the culture is terminated, and the result is recorded as a negative test. No further study is made of negative tests. If the result of the first-stage test is positive, the culture may be subjected to further study to verify the findings of the first stage.
- b *Second stage:* A transfer is made from positive cultures of the first-stage test to a second culture medium. This test stage emphasizes provision to reduce confusion of results due to growth effects of extraneous bacteria, commonly achieved by addition of selective inhibitory agents. (The Confirmed Test for coliforms meets these requirements. Lactose and fermentation vials are provided for demonstration of coliforms in the medium. Brilliant green dye and bile salts are included as inhibitory agents which tend to suppress growth of practically all kinds of noncoliform bacteria, but do not suppress growth of coliform bacteria when used as directed).

If result of the second-stange test is negative, the study of the culture is terminated, and the result is recorded as a negative test. A negative test here means that the positive results of the first-stage test were "false positive," due to one or more kinds of extraneous bacteria. A positive second-stage test is partial confirmation of the positive results obtained in the first-stage test; the culture may be subjected to final identification through application of still further testing procedures. In routine practice, most sample examinations are terminated at the end of the second stage, on the assumption that the result would be positive if carried to the third, and final stage. This practice should be followed only if adequate testing is done to demonstrate that the assumption is valid. Some workers recommend continuing at least 5% of all sample examinations to the third stage to demonstrate the reliability of the second-stage results

B Quantitative Aspects of Tests

1 These methods for determining bacterial numbers are based on the assumption that the bacteria can be separated from one another (by shaking or other means) resulting in a suspension of individual bacterial cells, uniformly distributed through the original sample when the primary inoculation is made.

2 Multiple dilution tube tests for quantitative determinations apply a Most Probable Number (MPN) technique. In this procedure one or more measured portions of each of a stipulated series of decreasing sample volumes is inoculated into the first-stage culture medium. Through decreasing the sample increments, eventually a volume is reached where only one cell is introduced into some tubes, and no cells are introduced into other tubes. Each of the several tubes of sample-inoculated first-stage medium is tested independently, according to the principles previously described, in the qualitative aspects of testing procedures.

3 The combination of positive and negative results is used in an application of probability mathematics to secure a single MPN value for the sample.

4 To obtain MPN values, the following conditions must be met:

  a The testing procedure must result in one or more tubes in which the test organism is demonstrated to be present; and

  b The testing procedure must result in one or more tubes in which the test organism is not demonstrated to be present.

5 The MPN value for a given sample is obtained through the use of MPN Tables. It is emphasized that the precision of an individual MPN value is not great when compared with most physical or chemical determinations.

6 Standard practice in water pollution surveys conducted by this organization, is to plant five tubes in each of a series of sample increments, in sample volumes decreasing at decimal intervals. For example, in testing known polluted waters, the initial sample inoculations might consist of 5 tubes each in volumes of 0.1, 0.01, 0.001, and 0.0001 ml, respectively. This series of sample volumes will yield determinate results from a low of 200 to a high of 1,600,000 organisms per 100 ml.

III LABORATORY BENCH RECORDS

A Features of a Good Bench Record Sheet

1 Provides complete identification of the sample.

2 Provides for full, day-by-day information about all tests performed on the sample.

3 Provides easy step-by-step record applicable to any portion of the sample.

4 Provides for recording of the quantitative result which will be transcribed to subsequent reports.

5 Minimizes the amount of writing by the analyst.

6 Identifies the analyst(s).

B There is no such thing as "standard" bench sheet for multiple tube tests; there are many versions of bench sheets. Some are prescribed by administrative authority (such as the Office of a State Sanitary Engineer); others are devised by laboratory or project personnel to meet specific needs.

C It is not the purpose of this discussion to recommend an "ideal" bench form; however, the form used in this training course manual is essentially similar to that used in certain research laboratories of this organization. The student enrolled in the course for which this manual is written should make himself thoroughly familiar with the bench sheet and its proper use. See Figure 1.

IV NOTES ABOUT WORKING PROCEDURES IN THE LABORATORY

A Each bacteriological examination of water by multiple dilution tube methods requires a considerable amount of manipulation; much is quite repetitious. Laboratory workers must develop and maintain good routine working habits, with constant alertness to guard against lapses into careless, slip-shod laboratory procedures and "short cuts" which only can lead to lowered quality of laboratory work.

B Specific attention is brought to the following by no means exhaustive, critical aspects of laboratory procedures in multiple dilution tube tests:

1 Original sample

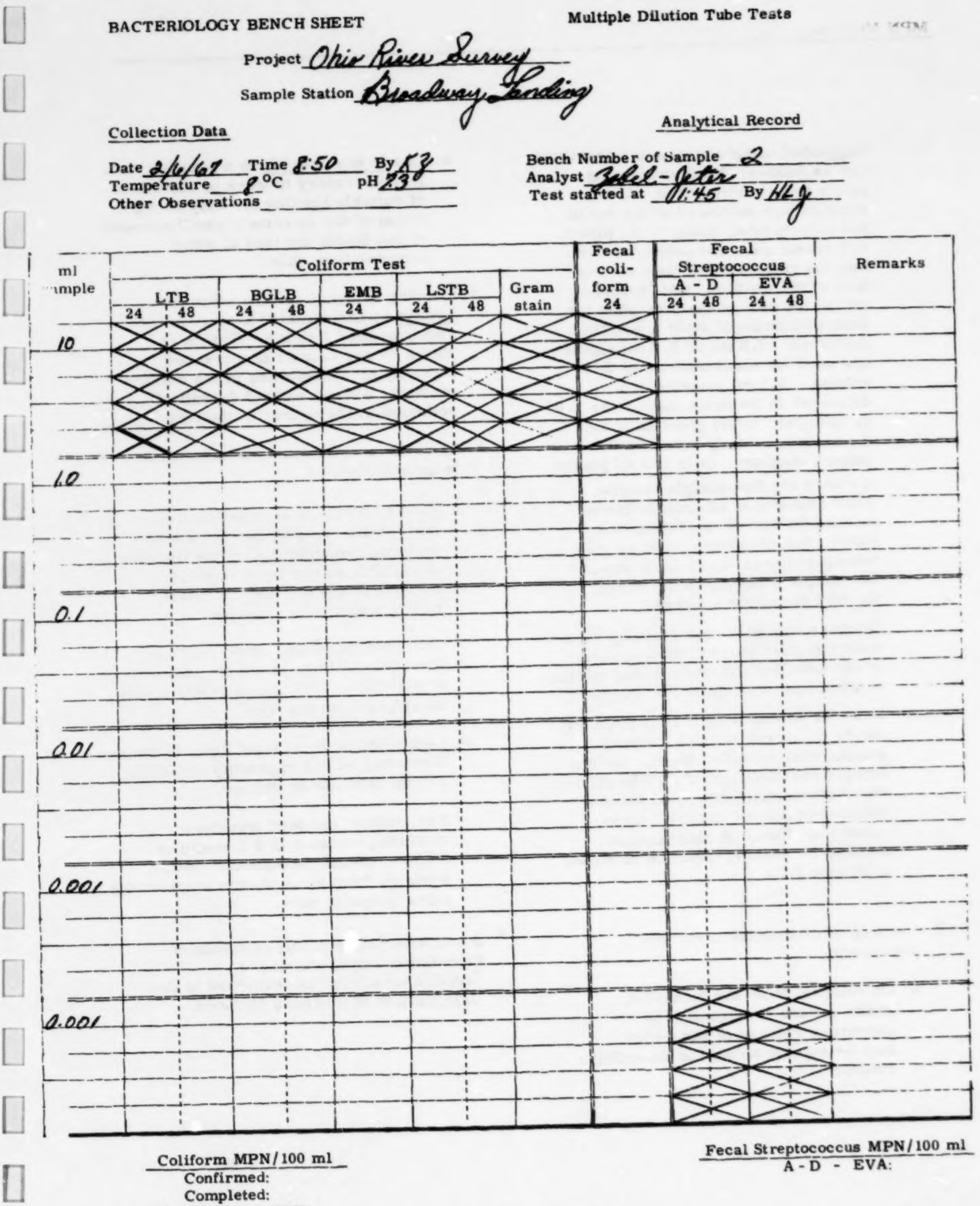
a Follow prescribed care and handling procedures before testing.

b Maintain absolute identification of sample at all stages in testing.

c Vigorously shake samples (and sample dilutions) before planting in culture media.

2 Sample measurement into primary culture medium

a Sample portions must be measured accurately into the culture medium for reliable quantitative tests to be made. Standard Methods prescribes that calibration errors should not exceed ± 2.5%.



BACTERIOLOGY BENCH SHEET

Multiple Dilution Tube Tests

Project Ohio River Survey

Sample Station Broadway Landing

Collection Data

Date 2/6/67 **Time** 8:50 **By** K J
Temperature 8 °C **pH** 7.3
Other Observations

Analytical Record

Bench Number of Sample 2
Analyst Zobel - Jeter
Test started at 11:45 **By** HLJ

ml mple	Coliform Test								Fecal coli- form	Fecal Streptococcus				Remarks
	LTB		BGLB		EMB	LSTB		Gram stain		A - D		EVA		
	24	48	24	48	24	24	48		24	24	48	24	48	
10														
1.0														
0.1														
0.01														
0.001														
0.001														

Coliform MPN/100 ml
Confirmed:
Completed:
Fecal Coliform MPN:

Fecal Streptococcus MPN/100 ml
A - D - EVA:

Figure 1. SAMPLE BENCH SHEET

Suggested sample measuring practices are as follows: Mohr measuring pipets are recommended. 10 ml samples are delivered at the top of the culture tube, using 10 ml pipets. 1.0 ml samples are delivered down into the culture tube, near the surface of the medium, and "touched off" at the side of the tube when the desired amount of sample has been delivered. 1.0 ml or 2.0 ml pipets are used for measurement of this volume. 0.1 ml samples are delivered in the same manner as 1.0 ml samples, using great care that the sample actually gets into the culture medium. Only 1.0 ml pipets are used for this sample volume. After delivery of all sample increments into the culture tubes, the entire rack of culture tubes may be shaken gently to carry down any of the sample adhering to the wall of the tube above the medium.

Workers should demonstrate by actual tests that the pipets and the technique in use actually delivers the rated volumes within the prescribed limits of error.

b Volumes as small as 0.1 ml routinely can be delivered directly from the sample with suitable pipets. Lesser sample volumes first should be diluted, with subsequent delivery of suitable volumes of diluted sample into the culture medium. A diagrammatic scheme for making dilutions is shown in Figure 2.

3 Reading of culture tubes for gas production

a On removal from the incubator, shake culture rack gently, to encourage release of gas which may be supersaturated in the culture medium.

b Gas in any quantity is a positive test. It is necessary to work in conditions of suitable lighting for easy recognition of the extremely small amounts of gas inside the tops of some fermentation vials.

4 Reading of liquid culture tubes for growth as indication of a positive test requires good lighting. Growth is shown by any amount of increased turbidity or opalescence in the culture medium, with or without deposit of sediment at the bottom of the tube.

5 Transfer of cultures with inoculation loops and needles

a Always sterilize inoculation loops and needles to glowing (white hot) in flame immediately before transfer of culture; do not lay it down or touch it to any non sterile object before making the transfer.

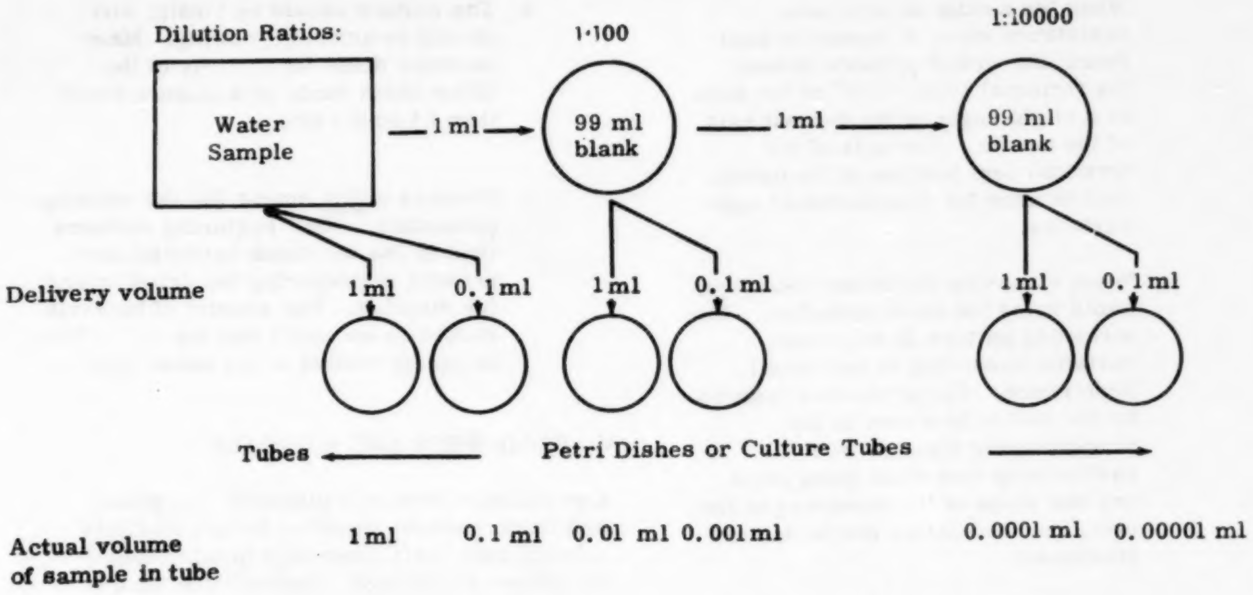
b After sterilization, allow sufficient time for cooling, in the air, to avoid heat-killing bacterial cells which will be gathered on the wire.

c Loops should be at least 3mm in inside diameter, with a capability of holding a drop of water or culture.

For routine standard transfers requiring transfer of 3 loopsful of culture, (Fecal Steptococci) many workers form three 3-mm loops on the same length of wire.

6 As an alternative to use of standard inoculation loops, the use of "applicator sticks" is described in the 14th Edition of Standard Methods.

Figure 2. PREPARATION OF DILUTIONS


Dilution Ratios:
1:100
1:10000
Water Sample
1 ml
99 ml blank
1 ml
99 ml blank
Delivery volume
1 ml
0.1 ml
1 ml
0.1 ml
1 ml
0.1 ml
Tubes
Petri Dishes or Culture Tubes
Actual volume of sample in tube
1 ml
0.1 ml
0.01 ml
0.001 ml
0.0001 ml
0.00001 ml

The applicator sticks are dry heat sterilized (autoclave sterilization is not acceptable because of possible release of phenols if the wood is steamed) and are used on a single-service basis. Thus, for every positive culture tube transferred, a new applicator stick is used.

This use of applicator sticks is particularly attractive in field situations where it is inconvenient or impossible to provide a gas burner suitable for sterilization of the inoculation loop. In addition, use of applicator sticks is favored in laboratories where room temperatures are significantly elevated by use of gas burners.

7 Streaking cultures on agar surfaces

a All streak-inoculations should be made without breaking the surface of the agar. Learn to use a light touch with the needle; however, many inoculation needles are so sharp that they are virtually useless in this respect. When the needle is platinum or platinum-iridium wire, it sometimes is beneficial to fuse the working tip into a small sphere. This can be done by momentary insertion of a well-insulated (against electricity) wire into a carbon arc, or some other extremely hot environment. The sphere should not be more than twice the diameter of the wire from which it is formed, otherwise it will be entirely too heat-retentive to be useful.

When the needle is nichrome resistance wire, it cannot be heat-fused; the writer prefers to bend the terminal 1/16 - 1/8" of the wire at a slight angle to the overall axis of the needle. The side of the terminal bent portion of the needle then is used for inoculation of agar surfaces.

b When streaking for colony isolation, avoid using too much inoculum. The streaking pattern is somewhat variable according to individual preference. The procedure favored by the writer is shown in the accompanying figure. Note particularly that when going from any one stage of the streaking to the next, the inoculation needle is heat-sterilized.

8 Preparation of cultures for Gram stain

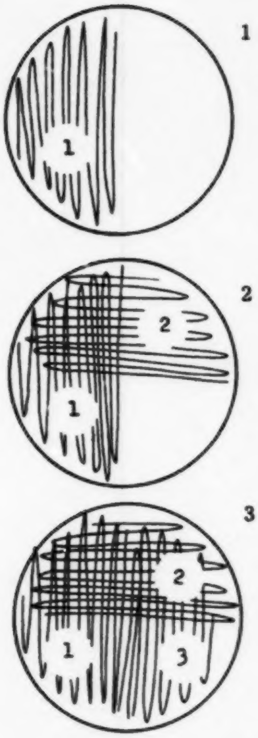
a The Gram stain always should be made from a culture grown on a nutrient agar surface (nutrient agar slants are used here) or from nutrient broth.

b The culture should be young, and should be actively growing. Many workers doubt the validity of the Gram stain made on a culture more than 24 hours old.

c Prepare a thin smear for the staining procedure. Most beginning workers tend to use too much bacterial suspension in preparing the dried smear for staining. The amount of bacteria should be so small that the [illegible] film is barely visible to the naked eye.

V EQUIPMENT AND SUPPLIES

Consolidated lists of equipment, supplies, and culture media required for all multiple dilution tube tests described in this outline are shown in Table 2. Quantitative information is not presented; this is variable - according to the extent of the testing procedure, the number of dilutions used, and the number of replicate tubes per dilution. It is noted that requirements for alternate procedures are fully listed and choices are made in accordance to laboratory preference.



1 a Flame-sterilize an inoculation needle and air-cool.

b Dip the tip of the inoculation needle into the bacterial culture being studied.

c Streak the inoculation needle tip lightly back and forth over half the agar surface, as in (1), avoiding scratching or breaking the agar surface.

d Flame-sterilize the inoculation needle and air-cool.

2 a Turn the Petri dish one-quarter-turn and streak the inoculation needle tip lightly back and forth over one-half the agar surface, working from area (1) into one-half the unstreaked area of the agar.

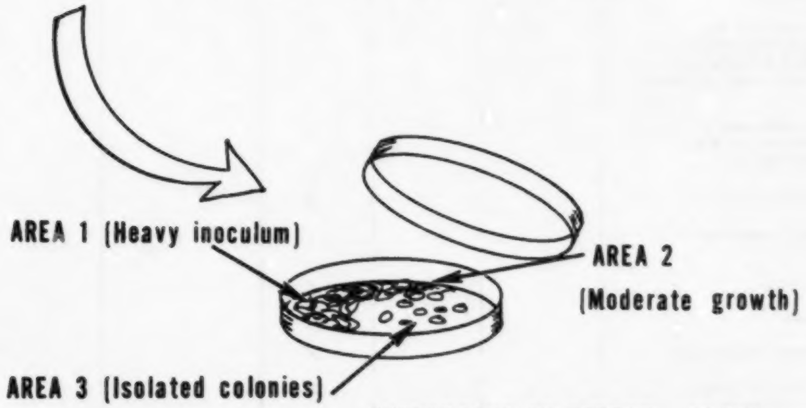
b Flame-sterilize the inoculation needle and air-cool.

3 a Turn the Petri dish one-quarter-turn and streak the inoculation needle tip lightly back and forth over one-half the agar surface, working from area (2) into area (3), the remaining unstreaked area.

b Flame-sterilize the inoculation needle and set it aside.

c Close the culture container and incubate as prescribed.

Figure 3. A SUGGESTED PROCEDURE FOR COLONY ISOLATION BY A STREAK-PLATE TECHNIQUE



APPEARANCE OF STREAK - PLATE
AFTER INCUBATION INTERVAL

TABLE 2. APPARATUS AND SUPPLIES FOR STANDARD FERMENTATION TUBE TESTS

Description of Item	Total Coliform Group			Fecal Coliforms
	Presumptive Test	Confirmed Test	Completed Test	(EC broth)
Lauryl tryptose broth or Lactose broth. 20 ml amounts of 1.5 × concentration medium, in 25 × 150 mm culture tubes with inverted fermentation vials, suitable caps.	X			
Lauryl tryptose broth or Lactose broth. 10 ml amounts of single strength medium in 20 × 150 mm culture tubes with inverted fermentation vials, suitable caps.	X		X	
Brilliant green lactose bile broth, 2% in 10 ml amounts, single strength, in 20 × 150 mm culture tubes with inverted fermentation vials, suitable caps.		X	X	
Eosin methylene blue agar, poured in 100 × 15 mm Petri dishes		X	X	
Endo Agar, poured in 100 × 15 mm dishes		X		
Nutrient agar slant, screw cap tube			X	
EC Broth, 10 ml amounts of single strength medium in fermentation tubes.				X
Culture tube racks, 10 × 5 openings; each opening to accept 25 mm diameter tubes.	X	X	X	X
Pipettes, 10 ml, Mohr type, sterile, in suitable cans.	X			
Pipettes, 2 ml (optional), Morh type, sterile, in suitable cans	X			
Pipettes, 1 ml, Mohr type, sterile in metal suitable cans	X			
Standard buffered dilution water, sterile, 99-ml amounts in screw-capped bottles.	X			
Gas burner, Bunsen type		X	X	X
Inoculation loop, loop 3mm diameter, of nichrome or platinum-iridium wire, 26 B & S gauge, in suitable holder. (or sterile applicator stick)		X	X	X
Inoculation needle, nichrome, or platinum-iridium wire, 26 B & S gauge, in suitable holder.		X	X	
Incubator, adjusted to 35 ± 0.5°C	X	X	X	
Waterbath incubator, adjusted to 44.5 ± 0.2°C.				X
Glass microscopic slides,			X	
Slide racks (optional)			X	
Gram-stain solutions, complete set			X	
Compound microscope, oil immersion lens, Abbe' condenser			X	
Basket for discarded cultures	X	X	X	X
Container for discarded pipettes	X			

Part 2

DETAILED TESTING PROCEDURES FOR MEMBERS OF THE COLIFORM GROUP BY MULTIPLE DILUTION TUBE METHODS

I SCOPE

A Tests Described

1 Presumptive Test

2 Confirmed Test

3 Completed Test

4 Fecal Coliform Test

B Form of Presentation

The Presumptive, Confirmed, and Completed Tests are presented as total, independent procedures. It is recognized that this form of presentation is somewhat repetitious, inasmuch as the Presumptive Test is preliminary to the Confirmed Test, and both the Presumptive Test and the Confirmed Test are preliminary to the Completed Test for total coliforms.

In using these procedures, the worker must know at the outset what is to be the stage at which the test is to be ended, and the details of the procedures throughout, in order to prevent the possibility of discarding gas-positive tubes before proper transfer procedures have been followed.

Thus, if the worker knows that the test will be ended at the Confirmed Test, he will turn at once to Section III, TESTING TO THE CONFIRMED TEST STAGE, and will ignore Sections II and IV.

The Fecal Coliform Test is described separately, in Section V, as an adjunct to the Confirmed Test and to the Completed Test.

II TESTING TO PRESUMPTIVE TEST STAGE

A First-Day Procedures

1 Prepare a laboratory data sheet for the sample. Record the following information: assigned laboratory number, source of sample, date and time of collection, temperature of the source, name of sample collector, date and time of receipt of sample in the laboratory. Also show the date and time of starting tests in the laboratory, name(s) of worker(s) performing the laboratory tests, and the sample volumes planted.

2 Label the tubes of lauryl tryptose broth required for the initial planting of the sample (Table 3). The label should bear three identifying marks. The upper number is the identification of the worker(s) performing the test (applicable to personnel in training courses), the number immediately below is the assigned laboratory number, corresponding with the laboratory record sheet. The lower number is the code to designate the sample volume and which tube of a replicate series is indicated.

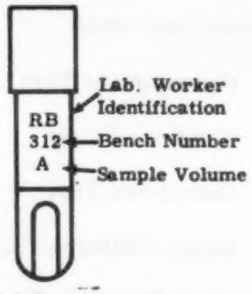
NOTE: Be sure to use tubes containing the correct concentrations of culture medium for the inoculum/tube volumes. (See the chapter on media and solutions for multiple dilution tube methods or refer to the current edition of Standard Methods for Water and Wastewater).

Table 3. SUGGESTED LABELING SCHEME FOR ORIGINAL CULTURES AND SUBCULTURES IN MULTIPLE DILUTION TUBE TESTS

	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Sample volume represented
Bench number Volume & tube	312 A	312 B	312 C	312 D	312 E	Tubes with 10 ml of sample
Bench number Volume & tube	312 a	312 b	312 c	312 d	312 e	Tubes with 1 ml of sample
Bench number Volume & tube	312 a	312 b	312 c	312 d	312 e	Tubes with 0.1 ml of sample
Bench number Volume & tube	312 1a	312 1b	312 1c	312 1d	312 1e	Tubes with 0.01 ml of sample
Bench number Volume & tube	312 2a	312 2b	312 2c	312 2d	312 2e	Tubes with 0.001 ml of sample

The labeling of cultures can be reduced by labeling only the first tube of each series of identical sample volumes in the initial planting of the sample. All subcultures from initial plantings should be labeled completely.

Typical Example



Tube of Culture Medium

3 Place the labeled culture tubes in an orderly arrangement in a culture tube rack, with the tubes intended for the largest sample volumes in the front row, and those intended for smaller volumes in the succeeding rows.

4 Shake the sample vigorously, approximately 25 times, in an arc of one foot within seven seconds and withdraw the sample portion at once.

5 Measure the predetermined sample volumes into the labeled tubes of lauryl tryptose broth, using care to avoid introduction of any bacteria into the culture medium except those in the sample.

a Use a 10 ml pipet for 10 ml sample portions, and 1 ml pipets for portions of 1 ml or less. Handle sterile pipets only near the mouthpiece, and protect the delivery end from external contamination. Do not remove the cotton plug in the mouthpiece as this is intended to protect the user from ingesting any sample.

b When using the pipet to withdraw sample portions, do not dip the pipet more than 1.25 cm (½ inch) into the sample; otherwise sample running down the outside of the pipet will make measurements inaccurate.

6 After measuring all portions of the sample into their respective tubes of medium, gently shake the rack of inoculated tubes to insure good mixing of sample with the culture medium. Avoid vigorous shaking, as air bubbles may be shaken into the fermentation vials and thereby invalidate the test.

7 Place the rack of inoculated tubes in the incubator at 35° ± 0.5°C for 24 ± 2 hours.

B 24-hour Procedures

1 Remove the rack of lauryl tryptose broth cultures from the incubator, and shake gently. If gas is about to appear in the fermentation vials, the shaking will speed the process.

2 Examine each tube carefully. Record, in the column "24" under LST on the laboratory data sheet, each tube showing gas in the fermentation vial as a positive (+) test and each tube not showing gas as a negative (-) test. GAS IN ANY QUANTITY IS A POSITIVE TEST.

3 Discard all gas-positive tubes of lauryl tryptose broth, and return all the gas-negative tubes to the 35°C incubator for an additional 24 ± 2 hours.

C 48-hour Procedures

1 Remove the rack of culture tubes from the incubator, read and record gas production for each tube.

2 Be sure to record all results under the 48-hour LTB column on the data sheet. Discard all tubes. The Presumptive Test is concluded at this point, and Presumptive coliforms per 100 ml can be computed according to the methods described elsewhere in this manual.

III TESTING TO CONFIRMED TEST STAGE

Note that the description starts with the sample inoculation and includes the Presumptive Test stage. The Confirmed Test preferred in Laboratories of this agency is accomplished by means of the brilliant green lactose bile broth (BGLB) and the acceptable alternate tests are mentioned in III F. In addition, the Fecal Coliform Test is included as an optional adjunct to the procedure.

A First-Day Procedures

1 Prepare a laboratory data sheet for the sample. Record the following information: assigned laboratory number, source of sample, date and time of collection, temperature of the source, name of sample collector, date and time of receipt of sample in the laboratory. Also show the date and time of starting tests in the laboratory name(s) of worker(s) performing the laboratory tests, and the sample volumes planted.

2 Label the tubes of lauryl tryptose broth required for the initial planting of the sample. The label should bear three identifying marks. The upper number is the identification of the worker(s) performing the test (applicable to personnel in training courses), the number immediately below is the assigned laboratory number, corresponding with the laboratory record sheet. The lower number is the code to designate the sample volume and which tube of a replicate series is indicated.

NOTE: If 10-ml samples are being planted, it is necessary to use tubes containing the correct concentration of culture medium. This has previously been noted in II A-2.

3 Place the labeled culture tubes in an orderly arrangement in a culture tube rack, with the tubes intended for the largest sample volumes in the front row, and those intended for smaller volumes in the succeeding rows.

4 Shake the sample vigorously, approximately 25 times, in an up-and-down motion.

5 Measure the predetermined sample volumes into the labeled tubes of lauryl tryptose broth, using care to avoid introduction of any bacteria into the culture medium except those in the sample.

a Use a 10-ml pipet for 10 ml sample portions, and 1-ml pipets for portions of 1 ml or less. Handle sterile pipets only near the mouthpiece, and protect the delivery end from external contamination. Do not remove the cotton plug in the mouthpiece as this is intended to protect the user from ingesting any sample.

b When using the pipet to withdraw sample portions, do not dip the pipet more than 1.25 cm (½ inch) into the sample; otherwise sample running down the outside of the pipet will make measurements inaccurate.

c When delivering the sample into the culture medium, deliver sample portions of 1 ml or less down into the culture tube near the surface of the medium. Do not deliver small sample volumes at the top of the tube and allow them to run down inside the tube; too much of the sample will fail to reach the culture medium.

d Prepare preliminary dilutions of samples for portions of 0.01 ml or less before delivery into the culture medium. See Table 1 for preparation of dilutions. NOTE: Always deliver diluted sample portions into the culture medium as soon as possible after preparation. The interval between preparation of dilution and introduction of sample into the medium never should be as much as 30 minutes.

6 After measuring all portions of the sample into their respective tubes of medium, gently shake the rack of inoculated tubes to insure good mixing of sample with the culture medium. Avoid vigorous shaking, as air bubbles may be shaken into the fermentation vials and thereby invalidate the test.

7 Place the rack of inoculated tubes in the incubator at 35° ± 0.5°C for 24 ± 2 hours.

B 24-hour Procedures

1 Remove the rack of lauryl tryptose broth cultures from the incubator, and shake gently. If gas is about to appear in the fermentation vials, the shaking will speed the process.

2 Examine each tube carefully. Record, in the column "24" under LST on the laboratory data sheet, each tube showing gas in the fermentation vial as a positive (+) test and each tube not showing gas as a negative (-) test. GAS IN ANY QUANTITY IS A POSITIVE TEST.

3 Retain all gas-positive tubes of lauryl tryptose broth culture in their place in the rack, and proceed.

4 Select the gas-positive tubes of lauryl tryptose broth culture for Confirmed Test procedures. Confirmed Test procedures may not be required for all gas-positive cultures. If, after 24-hours of incubation, all five replicate cultures are gas-positive for two or more consecutive sample volumes, then select the set of five cultures representing the smallest volume of sample in which all tubes were gas-positive. Apply Confirmed Test procedures to all these cultures and to any other gas-positive cultures representing smaller volumes of sample, in which some tubes were gas-positive and some were gas-negative.

5 Label one tube of brilliant green lactose bile broth (BGLB) to correspond with each tube of lauryl tryptose broth selected for Confirmed Test procedures.

6 Gently shake the rack of Presumptive Test cultures. With a flame-sterilized inoculation loop transfer one loopful of culture from each gas-positive tube to the corresponding tube of BGLB. Place each newly inoculated culture into BGLB in the position of the original gas-positive tube.

7 After making the transfers, the rack should contain some 24-hour gas-negative tubes of lauryl tryptose broth and the newly inoculated BGLB.

8 If the Fecal Coliform Test is included in the testing procedures, consult Section V of this part of the outline of testing procedures.

9 Incubate the 24-hour gas-negative BGLB tubes and any newly-inoculated tubes of BGLB an additional 24 ± 2 hours at 35° ± 0.5°C.

C 48-hour Procedures

1 Remove the rack of culture tubes from the incubator, read and record gas production for each tube.

2 Some tubes will be lauryl tryptose broth and some will be brilliant green lactose bile broth (BGLB). Be sure to record results from LTB under the 48-hour LTB column and the BGLB results under the 24-hour column of the data sheet.

3 Label tubes of BGLB to correspond with all (if any) 48-hour gas-positive cultures in lauryl tryptose broth. Transfer one loopful of culture from each gas-positive LTB culture to the correspondingly-labeled tube of BGLB. NOTE: All tubes of LTB culture which were negative at 24 hours and became positive at 48 hours are to be transferred The option described above for 24-hour cultures does not apply at 48 hours.

4 If the Fecal Coliform Test is included in the testing procedure, consult Section V of the part of the outline of testing procedures.

5 Incubate the 24-hour gas-negative BGLB tubes and any newly-inoculated tubes of BGLB 24 ± 2 hours at 35° ± 0.5°C.

6 Discard all tubes of LTB and all 24-hour gas-positive BGLB cultures.

D 72-hour Procedures

1 If any cultures remain to be examined, all will be BGLB. Some may be 24 hours old and some may be 48 hours old. Remove such cultures from the incubator, examine each tube for gas production, and record results on the data sheet.

2 Be sure to record the results of 24-hour BGLB cultures in the "24" column under BGLB and the 48-hour results under the "48" column of the data sheet.

3 Return any 24-hour gas-negative cultures for incubation 24 ± 2 hours at 35 ± 0.5°C.

4 Discard all gas-positive BGLB cultures and all 48-hour gas-negative cultures from BGLB.

5 It is possible that all cultural work and results for the Confirmed Test have been finished at this point. If so, codify results and determine Confirmed Test coliforms per 100 ml as described in the outline on use of MPN Tables.

E 96-hour Procedures

At most only a few 48-hour cultures in BGLB may be present. Read and record gas production of such cultures in the "48" column under BGLB on the data sheet. Codify results and determine Confirmed Test coliforms per 100 ml.

F Streak-plate methods for the Confirmed Test, using eosin methylene blue agar or Endo agar plates, are accepted procedures in Standard Methods. The worker who prefers to use one of these media in preference to BGLB (also approved in Standard Methods) is advised to refer to the current edition of "Standard Methods for the Examination of Water and Wastewater" for procedures.

IV TESTING TO COMPLETED TEST STAGE

(Note that this description starts with the sample inoculation and proceeds through the Presumptive and the Confirmed Test stages. In addition, the Fecal Coliform Test is referred to as an optional adjunct to the procedure.)

A First-Day Procedures

1 Prepare a laboratory data sheet for the sample. Record the following information: assigned laboratory number, source of sample, date and time of collection, temperature of the source, name of sample collector, date and time of receipt of sample in the laboratory. Also show the date and time of starting tests in the laboratory, name(s) of worker(s) performing the laboratory tests, and the sample volumes planted.

2 Label the tubes of lauryl tryptose broth required for the initial planting of the sample. The label should bear three identifying marks. The upper number is the identification of the worker(s) performing the test (applicable to personnel in training courses), the number immediately below is the assigned laboratory number, corresponding with the laboratory record sheet. The lower number is the code to designate the sample volume and which tube of a replicate series is indicated. Guidance on labeling for laboratory data number and identification of individual tubes is described elsewhere in this outline.

NOTE: If 10-ml samples are being plated, it is necessary to use tubes containing the correct concentration of culture medium. This has previously been noted elsewhere in this outline and referral is made to tables.

3 Place the labeled culture tubes in an orderly arrangement in a culture tube rack, with the tubes intended for the largest sample volumes in the front row, and those intended for smaller volumes in the succeeding rows.

4 Shake the sample vigorously, approximately 25 times, in an up-and-down motion.

5 Measure the predetermined sample volumes into the labeled tubes of lauryl tryptose broth, using care to avoid introduction of any bacteria into the culture medium except those in the sample.

a Use a 10-ml pipet for 10 ml sample portions, and 1-ml pipets for portions of 1 ml or less. Handle sterile pipets only near the mouthpiece, and protect the delivery end from external contamination. Do not move the cotton plug in the mouthpiece as this is intended to protect the user from ingesting any sample.

b When using the pipet to withdraw sample portions, do not dip the pipet more than 1.25 cm ($\frac{1}{2}$ inch) into the sample; otherwise sample running down the outside of the pipet will make measurements inaccurate.

c When delivering the sample into the culture medium, deliver sample portions of 1 ml or less down into the culture tube near the surface of the medium. Do not deliver small sample volumes at the top of the tube and allow them to run down inside the tube; too much of the sample will fail to reach the culture medium.

d Prepare preliminary dilutions of samples for portions of 0.01 ml or less before delivery into the culture medium. See Table 2 for preparation of dilutions. NOTE: Always deliver diluted sample portions into the culture medium as soon as possible after preparation. The interval between preparation of dilution and introduction of sample into the medium never should be as much as 30 minutes.

6 After measuring all portions of the sample into their respective tubes of medium, gently shake the rack of inoculated tubes to insure good mixing of sample with the culture medium. Avoid vigorous shaking, as air bubbles may be shaken into the fermentation vials and thereby invalidate the test.

7 Place the rack of inoculated tubes in the incubator at 35° ± 0.5°C for 24 ± 2 hours.

B 24-hour Procedures

1 Remove the rack of lauryl tryptose broth cultures from the incubator, and shake gently. If gas is about to appear in the fermentation vials, the shaking will speed the process.

2 Examine each tube carefully. Record, in the column "24" under LST on the laboratory data sheet, each tube showing gas in the fermentation vial as a positive (+) test and each tube not showing gas as a negative (-) test. GAS IN ANY QUANTITY IS A POSITIVE TEST.

3 Retain all gas-positive tubes of lauryl tryptose broth culture in their place in the rack, and proceed.

4 Select the gas-positive tubes of lauryl tryptose broth culture for the Confirmed Test procedures. Confirmed Test procedures may not be required for all gas-positive cultures. If, after 24-hours of incubation, all five replicate cultures are gas-positive for two or more consecutive sample volumes, then select the set of five cultures representing the smallest volume of sample in which all tubes were gas-positive. Apply Confirmed Test procedures to all these cultures and to any other gas-positive cultures representing smaller volumes of sample, in which some tubes were gas-positive and some were gas-negative.

5 Label one tube of brilliant green lactose bile broth (BGLB) to correspond with each tube of lauryl tryptose broth selected for Confirmed Test procedures.

6 Gently shake the rack of Presumptive Test cultures. With a flame-sterilized inoculation loop transfer one loopful of culture from each gas-positive tube to the corresponding tube of BGLB. Place each newly inoculated culture into BGLB in the position of the original gas-positive tube.

7 If the Fecal Coliform Test is included in the testing procedure, consult Section V of this outline for details of the testing procedure.

8 After making the transfer, the rack should contain some 24-hour gas-negative tubes of lauryl tryptose borth and the newly inoculated BGLB. Incubate the rack of cultures at 35°C ± 0.5°C for 24 ± 2 hours.

C 48-hour Procedures

1 Remove the rack of culture tubes from the incubator, read and record gas production for each tube.

2 Some tubes will be lauryl tryptose broth and some will be brilliant green lactose

bile broth (BGLB). Be sure to record results from LTB under the 48-hour LTB column and the BGLB results under the 24-hour column of the data sheet.

3 Label tubes of BGLB to correspond with all (if any) 48-hour gas-positive cultures in lauryl tryptose broth. Transfer one loopful of culture from each gas-positive LTB culture to the correspondingly-labeled tube of BGLB. NOTE: All tubes of LTB culture which were negative at 24 hours and became positive at 48 hours are to be transferred. The Option described above for 24-hour LTB cultures does not apply at 48 hours.

4 Incubate the 24-hour gas-negative BGLB tubes and any newly-inoculated tubes of BGLB 24 ± 2 hours at 35° ± 0.5°C. Retain all 24-hour gas-positive cultures in BGLB for further test procedures.

5 Label a Petri dish preparation of eosin methylene blue agar (EMB agar) to correspond with each gas-positive culture in BGLB.

6 Prepare a streak plate for colony isolation from each gas-positive culture in BGLB on the correspondingly-labeled EMB agar plate.

Incubate the EMB agar plates 24 ± 2 hours at 35° ± 0.5°C.

D 72-hour Procedures

1 Remove the cultures from the incubator. Some may be on BGLB; several EMB agar plates also can be expected.

2 Examine and record gas production results for any cultures in BGLB.

3 Retain any gas-positive BGLB cultures and prepare streak plate inoculations for colony isolation in EMB agar. Incubate the EMB agar plates 24 ± 2 hours at 35 ± 0.5° C. Discard the gas-positive BGLB cultures after transfer.

4 Reincubate any gas-negative BGLB cultures 24 ± 2 hours at 35° ± 0.5°C.

5 Discard all 48-hour gas-negative BGLB cultures.

6 Examine the EMB agar plates for the type of colonies developed thereon. Well-isolated colonies having a dark center (when viewed from the lower side, held toward a light) are termed "nucleated or fisheye" colonies, and are regarded as "typical" coliform colonies. A surface sheen may or may not be present on "typical" colonies. Colonies which are pink or opaque but are not nucleated are regarded as "atypical colonies." Other colony types are considered "noncoliform." Read and record results as + for "typical" (nucleated) colonies ± for "atypical" (non-nucleated pink or opaque colonies), and - for other types of colonies which might develop.

7 With plates bearing "typical" colonies, select at least one well-isolated colony and transfer it to a correspondingly-labeled tube of lactose broth and to an agar slant. As a second choice, select at least two "atypical" colonies (if typical colonies are not present) and transfer them to labeled tubes of lactose broth and to agar slants. As a third choice, in the absence of typical or atypical coliform-like colonies, select at least two well-isolated colonies representative of those appearing on the EMB plate, and transfer them to lactose broth and to agar slants.

8 Incubate all cultures transfered from EMB agar plates 24 ± 2 hours at 35 ± 0.5°C.

E 96-hour Procedures

1 Subcultures from the samples being studied may include: 48-hour tubes of BGLB, EMB agar plates, lactose broth tubes, and agar slant cultures.

2 If any 48-hour tubes of BGLB are present, read and record gas production in the "48" column under BGLB. From any gas-positive BGLB cultures prepare streak plate inoculations for colony isolation on EMB agar. Discard all tubes of BGLB, and incubate EMB agar plates 24 ± 2 hours at 35 ± 0.5°C.

3 If any EMB plates are present, examine and record results in the "EMB" column of the data sheet. Make transfers to agar slants and to lactose broth from all EMB agar plate cultures. In decreasing order of preference, transfer at least one typical colony, or at least two atypical colonies, or at least two colonies representative of those on the plate.

4 Examine and record results from the lactose broth cultures.

5 Prepare a Gram-stained smear from each of the agar slant cultures, as follows:

NOTE: Always prepare Gram stain from an actively growing culture, preferably about 18 hours old, and never more than 24 hours old. Failure to observe this precaution often results in irregular staining reactions.

a Thoroughly clean a glass slide to free it of any trace of oily film.

b Place one drop of distilled water on the slide.

c Use the inoculation needle to suspend a tiny amount of growth from the nutrient agar slant culture in the drop of water.

d Mix the thin suspension of cells with the tip of the inoculation needle, and allow the water to evaporate.

e "Fix" the smear by gently warming the slide over a flame.

f Stain the smear by flooding it for 1 minute with ammonium oxylate-crystal violet solution.

g Flush the excess dye solution off in gently running water.

h Flood the smear with Lugol's iodine for 1 minute.

i Wash the slide in gently running water.

j Decolorize the smear with acetone alcohol solution with gentle agitation for 10-30 seconds, depending upon extent of removal of crystal violet dye.

k Counterstain for 10 seconds with safranin solution, then wash in running water and gently blot dry with bibulous paper.

l Examine the slide under the microscope, using the oil immersion lens. Coliform bacteria are Gram-negative (pink to red color and nonspore-forming, rod-shaped cells, occurring singly, in pairs, or rarely in short chains.

m If typical coliform staining reaction and morphology are observed, record + in in the appropriate space under the "Gram Stain" column of the data sheet. If typical morphology and staining reaction are not observed, then mark it ± or -, and make suitable comment in the "remarks" column at the right-hand side of the data sheet.

n If spore-forming bacteria are observed, it will be necessary to repurify the culture from which the observations were made. Consult the instructor, or refer to Standard Methods, for procedures.

At this point, it is possible that all cultural work for the Completed Test has been finished. If so, codify results and determine Completed Test coliforms per 100 ml.

F 120-hour Procedures and following:

1. Any procedures to be undertaken from this point are "straggler" cultures on media already described, and requiring step-by-step methodology already given in detail. Such cultures may be on: EMB plates, agar slants, or lactose broth. The same time-and-temperature of incubation required for earlier studies applies to the "stragglers" as do the observations, staining reactions, and interpretation of results. On conclusion of all cultural procedures, codify results and determine Completed Test coliforms per 100 ml.

V FECAL COLIFORM TEST

A General Information

1 The procedure described is an elevated temperature test for fecal coliform bacteria.

2 Equipment required for the tests are those required for the Presumptive Test of Standard Methods, a water-bath incubator, and the appropriate culture media.

B Fecal Coliform Test with EC Broth

1 Sample: The test is applied to gas-positive tubes from the Standard Methods Presumptive Test (lauryl tryptose broth), in parallel with Confirmed Test procedures.

2 24-hour Operations. Initial procedures are the planting procedures described for the Standard Methods Presumptive Coliform test.

a After reading and recording gas-production on lauryl tryptose broth, temporarily retain all gas-positive tubes.

b Label a tube of EC broth to correspond with each gas-positive tube of lauryl tryptose broth. The option regarding transfer of only a limited number of tubes to the Confirmed Test sometimes can be applied here. However, the worker is urged to avoid exercise of this option until he has assured the applicability of the option by preliminary tests on the sample source.

c Transfer one loopful of culture from each gas-positive culture in lauryl tryptose broth to the correspondingly labeled tube of EC broth.

d Incubate EC broth tubes 24 ± 2 hours at 44.5 ± 0.2°C in a waterbath with water depth sufficient to come up at least as high as the top of the culture medium in the tubes. Place in waterbath as soon as possible after inoculation and always within 30 minutes after inoculation.

3 48-hour operations

a Remove the rack of EC cultures from the waterbath, shake gently, and record gas production for each tube. Gas in any quantity is a positive test.

b As soon as results are recorded, discard all tubes. (This is a 24-hour test for EC broth inoculations and not a 48-hour test.)

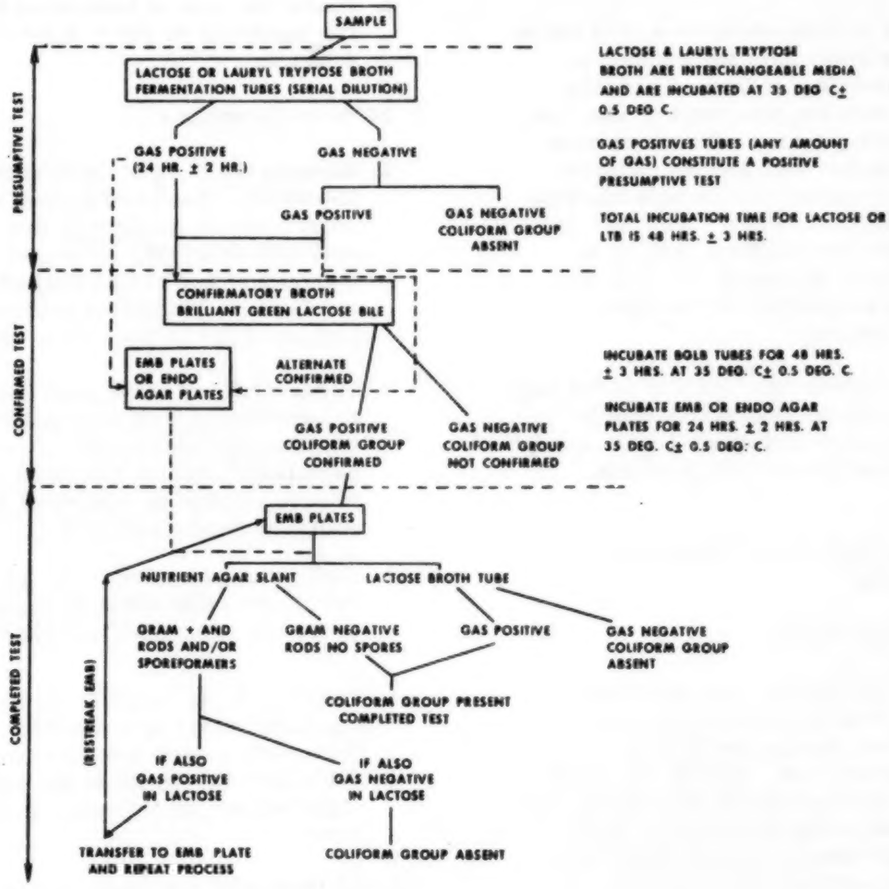
c Transfer any additional 48-hour gas-positive tubes of lauryl tryptose broth to correspondingly labeled tubes of EC broth. Incubate 24 ± 2 hours at 44.5 ± 0.2°C.

4 72-hour operations

a Read and record gas production for each tube. Discard all cultures.

b Codify results and determine fecal coliform count per 100 ml of sample.

TESTS FOR COLIFORM GROUP



SAMPLE
PRESUMPTIVE TEST
LACTOSE OR LAURYL TRYPTOSE BROTH FERMENTATION TUBES (SERIAL DILUTION)
GAS POSITIVE (24 HR. ± 2 HR.)
GAS NEGATIVE
GAS POSITIVE
GAS NEGATIVE COLIFORM GROUP ABSENT
CONFIRMED TEST
CONFIRMATORY BROTH BRILLIANT GREEN LACTOSE BILE
EMB PLATES OR ENDO AGAR PLATES
ALTERNATE CONFIRMED
GAS POSITIVE COLIFORM GROUP CONFIRMED
GAS NEGATIVE COLIFORM GROUP NOT CONFIRMED
COMPLETED TEST
EMB PLATES
NUTRIENT AGAR SLANT
LACTOSE BROTH TUBE
GRAM + AND RODS AND/OR SPOREFORMERS
GRAM NEGATIVE RODS NO SPORES
GAS POSITIVE
GAS NEGATIVE COLIFORM GROUP ABSENT
COLIFORM GROUP PRESENT COMPLETED TEST
(RESTREAK EMB)
IF ALSO GAS POSITIVE IN LACTOSE
IF ALSO GAS NEGATIVE IN LACTOSE
TRANSFER TO EMB PLATE AND REPEAT PROCESS
COLIFORM GROUP ABSENT

LACTOSE & LAURYL TRYPTOSE BROTH ARE INTERCHANGEABLE MEDIA AND ARE INCUBATED AT 35 DEG C± 0.5 DEG C.

GAS POSITIVES TUBES (ANY AMOUNT OF GAS) CONSTITUTE A POSITIVE PRESUMPTIVE TEST

TOTAL INCUBATION TIME FOR LACTOSE OR LTB IS 48 HRS. ± 3 HRS.

INCUBATE BGLB TUBES FOR 48 HRS. ± 3 HRS. AT 35 DEG. C± 0.5 DEG. C.

INCUBATE EMB OR ENDO AGAR PLATES FOR 24 HRS. ± 2 HRS. AT 35 DEG. C± 0.5 DEG: C.

Part 3

LABORATORY METHODS FOR FECAL STREPTOCOCCUS

(Day-By-Day Procedures)

I GENERAL INFORMATION

A The same sampling and holding procedures apply as for the coliform test.

B The number of fecal streptococci in water generally is lower than the number of coliform bacteria. It is good practice in multiple dilution tube tests to start the sample planting series with one sample increment larger than for the coliform test. For example: If a sample planting series of 1.0, 0.1, 0.01, and 0.001 ml is planned for the coliform test, it is suggested that a series of 10, 1.0, 0.1, and 0.01 ml be planted for the fecal streptococcus test.

C Equipment required for the test is the same as required for the Standard Methods Presumptive and Confirmed Tests, except for the differences in culture media.

II STANDARD METHODS (Tentative) PROCEDURES

A First-Day Operations

1 Prepare the sample data sheet and labeled tubes of azide dextrose broth in the same manner as for the Presumptive Test. NOTE: If 10-ml samples are included in the series, be sure to use a special concentration (ordinarily double-strength) of azide dextrose broth for these sample portions.

2 Shake the sample vigorously, approximately 25 times, in an up-and-down motion.

3 Measure the predetermined sample volumes into the labeled tubes of azide dextrose broth, using the sample measurement and delivery techniques used for the Presumptive Test.

4 Shake the rack of tubes of inoculated culture media, to insure good mixing of sample with medium.

5 Place the rack of inoculated tubes in the incubator at 35° ± 0.5° C for 24 ± 2 hours.

B 24-hour Operations

1 Remove the rack of tubes from the incubator. Read and record the results from each tube. Growth is a positive test with this test. Evidence of growth consists either of turbidity of the medium, a "button" of sediment at the bottom of the culture tube, or both.

2 Label a tube of ethyl violet azide broth to correspond with each positive culture of azide dextrose broth. It may be permissible to use the same confirmatory transfer option as described for the coliform Confirmed Test, in this outline.

3 Shake the rack of cultures gently, to resuspend cells which have settled out to the bottom of the culture tubes.

4 Transfer three loopfuls or use a wood applicator to transfer culture from each growth-positive tube of azide dextrose broth to the correspondingly labeled tube of ethyl violet azide broth.

5 As transfers are made, place the newly inoculated tubes of ethyl violet azide broth in a separate rack while returning the AD tubes to their former positions in the rack.

6 Return the rack, all azide dextrose broth tubes and newly-inoculated tubes of ethyl violet azide broth, to the incubator. Incubate 24 ± 2 hours at 35° ± 0.5°C.

C 48-hour Operations

1 Remove the rack of tubes from the incubator. Read and report results. Growth, either in azide dextrose broth or in ethyl violet azide broth, is a positive test. Be sure to report the results of the azide dextrose broth medium under the "48" column for that medium and the results of the ethyl violet azide broth cultures under the "24" column for that medium.

2 Any 48-hour growth-positive cultures of azide dextrose broth are to be transferred (as before) to ethyl violet azide broth. Discard all 48-hour growth-negative tubes of azide dextrose broth and all 24-hour growth-positive tubes of ethyl violet azide broth.

3 Re-incubate the 24-hour growth-negative Ethyl Violet azide tubes after again re-inoculating with their respective positive Azide Dextrose tubes and the newly-inoculated tubes of ethyl violet azide broth 24 ± 2 hours at 35° ± 0.5°C.

D 72-hour Operations

1 Read and report growth results of all tubes of ethyl violet azide broth.

2 Discard all growth-positive cultures and all 48-hour growth-negative cultures.

3 Reincubate any 24-hour growth-negative cultures in ethyl violet azide broth after reinoculating with their respective positive azide dextrose tubes for an additional 24 ± 2 hours at 35° ± 0.5°C.

E 96-hour Operations

1 Read and report growth results of any remaining tubes of ethyl violet azide broth.

2 Codify results and determine fecal streptococci per 100 ml.

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This outline was prepared by H. L. Jeter, Chief, Program Support Training Branch, USEPA, Cincinnati, Ohio 45268

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DETAILED MEMBRANE FILTER METHODS

I BASIC PROCEDURES

A Introduction

Successful application of membrane filter methods requires development of good routine operational practices. The detailed basic procedures described in this Section are applicable to all membrane filter methods in water bacteriology for filtration, incubation, colony counting, and reporting of results. In addition, equipment and supplies used in membrane filter procedures described here are not repeated elsewhere in this text in such detail.

Workers using membrane filter methods for the first time are urged to become thoroughly familiar with these basic procedures and precautions.

B General Supplies and Equipment List

Table 1 is a check list of materials.

C "Sterilizing" Media

Set tubes of freshly prepared medium in a boiling waterbath for 10 minutes. This method suffices for medium in tubes up to 25 × 150 mm. Frequent agitation is needed with media containing agar.

Alternately, coliform media can be directly heated on a hotplate to the first bubble of boiling. Stir the medium frequently if direct heat is used, to avoid charring the medium.

Do not sterilize in the autoclave.

D General Laboratory Procedures with Membrane Filters

1 Prepare data sheet

Minimum data required are: sample identification, test performed including media and methods, sample filtration volumes, and the bench numbers assigned to individual membrane filters.

2 Disinfect the laboratory bench surface.

Use a suitable disinfectant solution and allow the surface to dry before proceeding.

3 Set out sterile culture containers in an orderly arrangement.

4 Label the culture containers.

Numbers correspond with the filter numbers shown on the data sheet.

5 Place one sterile absorbent pad* in each culture container, unless an agar medium is being used.

Use sterile forceps for all manipulations of absorbent pads and membrane filters. Forceps sterility is maintained by storing the working tips in about 1 inch of methanol or ethanol. Because the alcohol deteriorates the filter, dissipate it by burning before using the forceps. Avoid heating the forceps in the burner as hot metal chars the filter.

*When an agar medium is used, absorbent pads are not used. The amount of medium should be sufficient to make a layer approximately 0.3 cm (1/8 inch) deep in the culture container. In the 50 mm plastic culture containers this corresponds to approximately 6-8 ml of culture medium.

NOTE: Mention of commercial products and manufacturers does not imply endorsement by the Office Of Water Programs, Environmental Protection Agency.

Table 1. EQUIPMENT, SUPPLIES AND MEDIA

	Total Coliforms					
Item	M-Endo Broth	L. E. S. Coliform	Delayed Coliform	Fecal Coliform	Fecal Streptococcus	Verified Tests
Funnel unit assemblies	X	X	X	X	X	
Ring stand, with about a 3" split ring, to support the filtration funnel	X	X	X	X	X	
Forceps, smooth tips, type for MF work	X	X	X	X	X	
Methanol, in small wide-mouthed bottles, about 20 ml for sterilizing forceps	X	X	X	X	X	
Suction flasks, glass, 1 liter, mouth to fit No. 8 stopper	X	X	X	X	X	
Rubber tubing, 60-90 cm (2-3 feet), to connect suction flask to vacuum services, latex rubber .5 cm (3/16") I.D. by .2 cm wall (3/16 x 3/32 inch):	X	X	X	X	X	
Pinch clamps strong enough for tight compression of rubber tubing above	X	X	X	X	X	
Pipettes, 10 ml, graduated, Mohr type, sterile, dispense 10 per can per working space per day. (Resterilize daily to meet need).	X	X	X	X	X	
Pipettes, 1 ml, graduated, Mohr type, sterile, dispense 24 per can per working space per day. (Resterilize daily to meet need).	X	X	X	X	X	
Pipette boxes, sterile, for 1 ml and 10 ml pipettes (sterilize above pipettes in these boxes).	X	X	X	X	X	
Cylinders, 100 ml graduated, sterile, (resterilize daily to meet need).	X	X	X	X	X	
Jars, to receive used pipettes	X	X	X	X	X	
Gas burner, Bunsen or similar laboratory type	X	X	X	X	X	X
Wax pencils, red, suitable for writing on glass	X	X	X	X	X	
Sponge in dilute iodine, to disinfect the desk tops	X	X	X	X	X	
Membrane filters (white, grid marked, sterile,and suitable pore size for microbiological analysis of water)	X	X	X	X	X	
Absorbent pads for nutrient, (47 mm in diameter), sterile, in units of 10 pads per package. Not required if medium contains agar.	X	X	X	X	X	
Petri dishes, disposable, plastic, 50 × 12 mm, sterile	X	X	X	X	X	
Waterbath incubator 44.5 ± 0.2°C				X		
Vegetable crispers, or cake boxes, plastic, with tight fitting covers, for membrane filter incubations	X	X	X		X	
Fluorescent lamp, with extension cord.	X	X	X	X	X	X
Ring stand, with clamps, utility type	X	X	X	X	X	

Table 1. EQUIPMENT, SUPPLIES AND MEDIA (Cont'd)

Item	Total Coliforms			Fecal Coliform	Fecal Streptococcus	Verified Tests
	M-Endo Broth	L. E. S. Coliform	Delayed Coliform			
ve:						
Hand tally, single unit acceptable, hand or desk type (optional)	X	X	X	X	X	
Stereoscopic (dissection) microscope, magnification of 10X or 15X, preferable binocular wide field type	X	X	X	X	X	X
Bacteriological inoculating needle						X
Wire racks for culture tubes, 10 openings by five openings preferred, dimensions overall approximately .5 cm x 30 cm (6" x 12")						X
Phenol Red Lactose Broth in 16 X 150 mm fermentation tubes with metal caps, 10 ml per tube						X
Eosin Methylene Blue Agar (Levine) in petri plates, prepared ready for use						X
Nutrient agar slants, in screw capped tubes, 16 × 126 mm						X
Gram stain solutions, 4 solutions per complete set						X
Microscope, compound, binocular, with oil immersion lens, microscope lamp and immersion oil						X
Microscope slides, new, clean.						X
Water proof plastic bags for fecal coliform culture dish incubation				X		
M-Endo medium, MF dehydrated medium in 25 × 95 mm flat bottomed screw-capped glass vials, 1.44 g per tube, sufficient for 30 ml of medium	X		X			
Ethanol, 95% in small bottles or screw-capped tubes, about 20 ml per tube	X	X	X			
Sodium benzoate solution, 12% aqueous, in 25 × 150 mm screw-capped tubes, about 10 ml per tube			X			
L. E. S. Endo Agar MF, dehydrated M-Endo medium, 0.36 g per 25 × 95 mm flat bottomed screw-capped glass vial, plus 0.45 g agar, for 30 ml		X				
Lactose Lauryl Sulfate Tryptose Broth in 25 × 150 mm test tube without included gas tube, about 25 ml, for enrichment in L. E. S. method		X				

Table 1. EQUIPMENT, SUPPLIES AND MEDIA (Cont'd)

	Total Coliforms					
Item	M-Endo Broth	L. E. S. Coliform	Delayed Coliform	Fecal Coliform	Fecal Streptococcus	Verified Test
M-FC Broth for fecal coliform, dehydrated medium in 25 × 95 mm flat bottomed screw-capped glass vials, 1.11 g per tube, sufficient for 30 ml of culture medium				X		
Rosolic acid, 1% solution, in 0.2N NaOH, in 25 × 150 mm flat bottomed screw-capped tubes, about 5 ml per tube, freshly prepared				X		
KF Agar, dehydrated medium in 25 X 150 mm screw-capped tubes, sufficient for 30 ml, 2.3g per tube					X	
Dilution bottles, 180 ml, preferable boro-silicate glass, with screw-cap (or rubber stopper protected by paper), each containing 99 ml of sterile phosphate buffered distilled water	X	X	X	X	X	
Electric hot plate surface	X	X	X	X	X	
Beakers, 400 - 600 ml (for water-bath in preparation of membrane filter culture media)	X	X	X	X	X	
Crucible tongs, to be used at electric hot plates, for removal of hot tubes of culture media for boiling waterbath	X	X	X	X	X	

6 Deliver enough culture medium to saturate each absorbent pad, using a sterile pipette.

Exact quantities cannot be stated because pads and culture containers vary. Sufficient medium should be applied so that when the culture container is tipped, a good-sized drop of culture medium freely drains out of the absorbent pad.

7 Organize supplies and equipment for convenient sample filtration. In training courses, laboratory instructors will suggest useful arrangements; eventually the individual will select a system of bench-top organization most suited to his own needs. The important point in any arrangement is to have all needed equipment and supplies conveniently at hand, in such a pattern as to minimize lost time in useless motions.

8 Lay a sterile membrane filter on the filter holder, grid-side up, centered over the porous part of the filter support plate.

Membrane filters are extremely delicate and easily damaged. For manipulation, the sterile forceps should always grasp the outer part of the filter disk, outside the part of the filter through which the sample passes.

9 Attach the funnel element to the base of the filtration unit.

To avoid damage to the membrane filter, locking forces should only be applied at the locking arrangement. The funnel element never should be turned or twisted while being seated and locked to the lower element of the filter holding unit. Filter holding units featuring a bayonet joint and locking ring to join the upper element to the lower element require special care on the part of the operator. The locking ring should be turned sufficiently to give a snug fit, but should not be tightened excessively.

10 Shake the sample thoroughly.

11 Measure sample into the funnel with vacuum turned off.

The primary objectives here are: 1) accurate measurement of sample; and 2) optimum distribution of colonies on the filter after incubation. To meet these objectives, methods of measurement and dispensation to the filtration assembly are varied with different sample filtration volumes.

a With samples greater than 20 ml, measure the sample with a sterile graduated cylinder and pour it into the funnel. It is important to rinse this graduate with sterile buffered distilled water to preclude the loss of excessive sample volume. This should be poured into the funnel.

b With samples of 10 ml to 20 ml, measure the sample with a sterile 10 ml or 20 ml pipette, and pipette on a dry membrane in the filtration assembly.

c With samples of 2 ml to 10 ml, pour about 20 ml of sterile dilution water into the filtration assembly, then measure the sample into the sterile buffered dilution water with a 10 ml sterile pipette.

d With samples of 0.5 to 2 ml, pour about 20 ml of sterile dilution water into the funnel assembly, then measure the sample into the sterile dilution water in the funnel with a 1 ml or a 2 ml pipette.

e If a sample of less than 0.5 ml is to be filtered, prepare appropriate dilutions in sterile dilution water, and proceed as applicable in item c or d above.

When dilutions of samples are needed, always make the filtrations as soon as possible after dilution of the sample; this never should exceed

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30 minutes. Always shake sample dilutions thoroughly before delivering measured volumes.

12 Turn on the vacuum.

Open the appropriate spring clamp or valve, and filter the sample.

After sample filtration a few droplets of sample usually remain adhered to the funnel walls. Unless these droplets are removed, the bacteria contained in them will be a source of contamination of later samples. (In laboratory practice the funnel unit is not routinely sterilized between successive filtrations of a series). The purpose of the funnel rinse is to flush all droplets of a sample from the funnel walls to the membrane filter. Extensive tests have shown that with proper rinsing technique, bacterial retention on the funnel walls is negligible.

13 Rinse the sample through the filter.

After all the sample has passed through the membrane filter, rinse down the sides of the funnel walls with at least 20 ml of sterile dilution water. Repeat the rinse twice after all the first rinse has passed through the filter. Cut off suction on the filtration assembly.

14 Remove the funnel element of the filter holding unit.

If a ring stand with split ring is used, hang the funnel element on the ring; otherwise, place the inverted funnel element on the inner surface of the wrapping material. This requires care in opening the sterilized package, but it is effective as a protection of the funnel ring from contamination.

15 Take the membrane filter from the filter holder and carefully place it, grid-side up on the medium.

Check that no air bubbles have been trapped between the membrane filter and the underlying absorbent pad or agar. Relay the membrane if necessary.

16 Place in incubator after finishing filtration series.

Invert the containers. The immediate atmosphere of the incubating membrane filter must be at or very near 100% relative humidity.

17 Count colonies which have appeared after incubating for the prescribed time.

A stereoscopic microscope magnifying 10-15 times and careful illumination give best counts.

For reporting results, the computation is:

$$\text{bacteria/100 ml} = \frac{\text{No. colonies counted} \times 100}{\text{Sample volume filtered in ml}}$$

Example:

A total of 36 colonies grew after filtering a 10 ml sample. The number reported is:

$$\frac{36 \text{ colonies}}{10 \text{ ml}} \times 100 = 360 \text{ per } 100 \text{ ml}$$

Report results to two significant figures.

Example:

A total of 40 colonies grew after filtering a 3 ml sample.

This calculation gives:

$$\frac{40 \text{ colonies}}{3 \text{ ml}} \times 100 = 1333.33 \text{ per } 100 \text{ ml}$$

But the number reported should be 1300 per 100 ml.

II MF LABORATORY TESTS FOR COLIFORM GROUP

A Standard Coliform Test (Based on M-Endo Broth MF)

1 Culture medium

a M-Endo Broth MF Difco 0749-02 or the equivalent BBL M-Coliform Broth 01-494

Preparation of Culture Medium (M-Endo Broth) for Standard MF Coliform Test

Yeast extract	1.5 g
Casitone or equivalent	5.0 g
Thiopeptone or equivalent	5.0 g
Tryptose	10.0 g
Lactose	12.5 g
Sodium desoxycholate	0.1 g
Dipotassium phosphate	4.375 g
Monopotassium phosphate	1.375 g
Sodium chloride	5.0 g
Sodium lauryl sulfate	0.05 g
Basic fuchsin (bacteriological)	1.05 g
Sodium sulfite	2.1 g
Distilled water (containing 20.0 ml ethanol)	1000 ml

This medium is available in dehydrated form and it is recommended that the commercially available medium be used in preference to compounding the medium of its individual constituents.

To prepare the medium for use, suspend the dehydrated medium at the rate of 48 grams per liter of water containing ethyl alcohol at the rate of 20 ml per liter.

As a time-saving convenience, it is recommended that the laboratory worker preweigh the dehydrated medium in closed tubes for several days, or even weeks, at one operation. With this system, a large number of increments of dehydrated medium (e.g., 1.44 grams), sufficient for some convenient (e.g., 30 ml) volume of finished culture medium are weighed and dispensed into screw-capped culture tubes, and stored until needed. Storage should preferably be in a darkened disiccator.

A supply of distilled water containing 20 ml stock ethanol per liter can be maintained.

When the medium is to be used, it is reconstituted by adding 30 ml of the distilled water-ethanol mixture per tube of pre-weighed dehydrated culture medium.

b Medium is "sterilized" as directed in I, C.

c Finished medium can be retained up to 96 hours if kept in a cool, dark place. Many workers prefer to reconstitute fresh medium daily.

2 Filtration and incubation procedures are as given in I, D.

Special instructions:

a For counting, use the wide field binocular dissecting microscope, or simple lens. For illumination, use a light source perpendicular to the plane of the membrane filter. A small fluorescent lamp is ideal for the purpose.

b Coliform colonies have a "metallic" surface sheen under reflected light which may cover the entire colony, or it may appear only in the center. Non-coliform colonies range from colorless to pink, but do not have the characteristic sheen.

c Record the colony counts on the data sheet, and compute the coliform count per 100 ml of sample.

B Standard Coliform Tests (Based on L. E. S. Endo Agar)

The distinction of the L. E. S. count is a two hour enrichment incubation on LST broth. M-Endo L. E. S. medium is used as agar rather than the broth.

1 Preparation of culture medium (L. E. S. Endo Agar) for L. E. S. coliform test

a Formula from McCarthy, Delaney, and Grasso (2)

Bacto-Yeast Extract	1.2 g
Bacto-Casitone	3.7 g
Bacto-Thiopeptone	3.7 g
Bacto-Tryptose	7.5 g
Bacto-Lactose	9.4 g
Dipotassium phosphate	3.3 g
Monopotassium phosphate	1.0 g
Sodium chloride	3.7 g
Sodium desoxycholate	0.1 g
Sodium lauryl sulfate	0.05 g
Sodium sulfite	1.6 g
Bacto-Basic fuchsin	0.8 g
Agar	15 g
Distilled water (containing 20 ml ethyl alcohol)	1000 ml

b To rehydrate the medium, suspend 51 grams in the water-ethyl alcohol solution.

c Medium is "sterilized" as directed in I, C.

d Pour 4-6 ml of freshly prepared Agar into the smaller half of the container. Allow the medium to cool and solidify.

2 Procedures for filtration and incubation

a Lay out the culture dishes in a row or series of rows as usual. Place these with the upper (lid) or top side down.

b Place one sterile absorbent pad in the larger half of each container (lid). Use sterile forceps for all manipulations of the pads. (Agar occupies smaller half or bottom).

c Using a sterile pipette, deliver enough single strength lauryl sulfate tryptose broth to saturate the pad only. Avoid excess medium.

d Follow general procedures for filtering in I, D. Place filters on pad with lauryl sulfate tryptose broth.

e Upon completion of the filtrations, invert the culture containers and incubate at 35°C for 1 1/2 to 2 hours.

3 2-hour procedures

a Transfer the membrane filter from the enrichment pad in the upper half to the agar medium in the lower half of the container. Carefully roll the membrane onto the agar surface to avoid trapping air bubbles beneath the membrane.

b Removal of the used absorbent pad is optional.

c The container is inverted and incubated 22 hours ± 2 hours ± 0.5°C.

4 Counting procedures are as in I, D.

5 L. E. S. Endo Agar may be used as a single-stage medium (no enrichment step) in the same manner as M-Endo Broth, MF.

C Delayed Incubation Coliform Test

This technique is applicable in situations where there is an excessive delay between sample collection and plating. The procedure is unnecessary when the interval between sample collection and plating is within acceptable limits.

1 Preparation of culture media for delayed incubation coliform test

a Preservative media M-Endo Broth base

To 30 ml of M-Endo Broth MF prepared in accordance with directions in II, A, 1 of this outline, add 1.0 ml of a sterile 12% aqueous solution of sodium benzoate.

L. E. S. MF Holding Medium-Coliform: Dissolve 12.7 grams in 1 liter of distilled water. No heating is necessary. Final pH 7.1 ± 0.1. This medium contains sodium benzoate.

b Growth media

M-Endo Broth MF is used, prepared as described in II, A, 1 earlier in this outline. Alternately, L. E. S. Endo Medium may be used.

2 General filtration followed is in I, D.

Special procedures are:

a Transfer the membrane filter from the filtration apparatus to a pad saturated with benzoated M-Endo Broth.

b Close the culture dishes and hold in a container at ambient temperature. This may be mailed or transported to a central laboratory. The mailing or transporting tube should contain accurate transmittal data sheets which correspond to properly labeled dishes.

Transportation time, in the case of mailed containers, should not exceed three days to the time of reception by the testing laboratory.

c On receipt in the central laboratory, unpack mailing carton, and lay out the culture containers on the laboratory bench.

d Remove the tops from the culture containers. Using sterile forceps, remove each membrane and its absorbent pad to the other half of the culture container.

e With a sterile pipette or sterile absorbent pad, remove preservative medium from the culture container.

f Place a sterile absorbent pad in each culture container, and deliver enough freshly prepared M-Endo Broth to saturate each pad.

g Using sterile forceps, transfer the membrane to the new absorbent pad containing M-Endo Broth. Place the membrane carefully to avoid entrapment of air between the membrane and the underlying absorbent pad. Discard the absorbent pad containing preservative medium.

h After incubation of 20 ± 2 hours at 35° C, count colonies as in the above section A, 2.

i If L. E. S. Endo Agar is used, the steps beginning with (e) above are omitted; and the membrane filter is removed from the preservative medium and transferred to a fresh culture container with L. E. S. Endo Agar, incubated, and colonies counted in the usual way.

D Verified Membrane Filter Coliform Test

This procedure applies to identification of colonies growing on Endo-type media used for determination of total coliform counts. Isolates from these colonies are studied for gas production from lactose and typical coliform morphology. In effect, the procedure corresponds with the Completed Test stage of the multiple fermentation tube test for coliforms.

Procedure:

1 Select a membrane filter bearing several well-isolated coliform-type colonies.

2 Using sterile technique, pick all colonies in a selected area with the inoculation needle, making transfers into tubes of phenol red lactose broth (or lauryl sulfate tryptose lactose

broth). Using an appropriate data sheet record the interpretation of each colony, using, for instance, "C" for colonies having the typical color and sheen of coliforms; "NC" for colonies not conforming to coliform colony appearance on Endotype media.

3 Incubate the broth tubes at 35° C ± 0.5°C.

4 At 24 hours:

a Read and record the results from the lactose broth fermentation tubes. The following code is suggested:

Code	
O	No indication of acid or gas production, either with or without evidence of growth.
A	Evidence of acid but not gas (applies only when a pH indicator is included in the broth medium)
G	Growth with production of gas. If pH indicator is used, use symbol AG to show evidence of acid. Gas in any quantity is a positive test.

b Tubes not showing gas production are returned to the 35° C incubator.

c Gas-positive tubes are transferred as follows:

1) Prepare a streak inoculation on EMB agar for colony isolation, and using the same culture.

2) Inoculate a nutrient agar slant.

3) Incubate the EMB agar plates and slants at 35° C ± 0.5°C.

5 At 48 hours:

a Read and record results of lactose broth tubes which were negative at 24 hours and were returned for further incubation.

b Gas-positive cultures are subjected to further transfers as in 4c. Gas-negative cultures are discarded without further study; they are coliform- negative.

c Examine the cultures transferred to EMB agar plates and to nutrient agar slants, as follows:

1) Examine the EMB agar plate for evidence of purity of culture; if the culture represents more than one colony type, discard the nutrient agar culture and reisolate each of the representative colonial types on the EMB plate and resume as with 4c for each isolation. If purity of culture appears evident, continue with c (2) below.

2) Prepare a smear and Gram stain from each nutrient agar slant culture. The Gram stain should be made on a culture not more than 24 hours old. Examine under oil immersion for typical coliform morphology, and record results.

6 At 72 hours:

Perform procedures described in 5c above, and record results.

7 Coliform colonies are considered verified if the procedures demonstrate a pure culture of bacteria which are gram negative nonspore-forming rods and produce gas from lactose at 35° C within 48 hours.

E Fecal Coliform Count (Based on M-FC Broth Base)

The count depends upon growth on a special medium at 44.5 ± 0.2°C.

1 Preparation of Culture Medium (M-FC Broth Base) for Fecal Coliform Count

a **Composition**

Tryptose	10.0 g
Proteose Peptone No. 3	5.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Lactose	12.5 g
Bile salts No. 3	1.5 g
Rosolic acid* (Allied Chemical)	10.0 ml
Aniline blue (Allied Chemical)	0.1 g
Distilled water	1000 ml

b To prepare the medium dissolve 37.1 grams in a liter of distilled water which contains 10 ml of 1% rosolic acid (prepared in 0.2 N NaOH).

Fresh solutions of rosolic acid give best results. Discard solutions which have changed from dark red to orange.

c To sterilize, heat to boiling as directed in I, C.

d Prepared medium may be retained up to 4 days in the dark at 2-8° C.

2 Special supplies

Small water proof plastic sacks capable of being sealed against water with capacity of 3 to 6 culture containers.

3 Filtration procedures are as given in I, D.

4 Elevated temperature incubation

a Place fecal coliform count membranes at 44.5 ± 0.2°C as rapidly as possible. Filter membranes for fecal coliform counts consecutively and immediately place them in their culture containers. Insert as many as six culture containers all oriented in the same way (i.e., all grid sides facing the same direction) into the sacks and seal. Tear off the perforated top, grasp the side wires, and twirl the sack to roll the open end inside the folds of sack. Then submerge the sacks with culture containers inverted beneath the surface of a 44.5 ± 0.2°C waterbath.

b Incubate for 22 ± 2 hours.

5 Counting procedures

Examine and count colonies as follows:

a Use a wide field binocular dissecting microscope with 5 - 10X magnification.

b Low angle lighting from the side is advantageous.

c Fecal coliform colonies are blue, generally 1-3 mm in diameter.

d Record the colony counts on the data sheet, and report the fecal coliform count per 100 ml of sample. (I, D, 17 illustrates method)

III TESTS FOR FECAL STREPTOCOCCAL GROUP-MEMBRANE FILTER METHOD

A 48 hour incubation period on a choice of two different media, giving high selectivity for fecal streptococci, are the distinctive features of the tests.

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*Prepare 1% solution of rosolic acid in 0.2 N NaOH. This dye is practically insoluble in water.

A Test for Members of Fecal Streptococcal Group based on KF-Agar

1 Preparation of the culture medium

a Formula: (The dehydrated formula of Bacto 0496 is shown, but equivalent constituents from other sources are acceptable). Formula is in grams per liter of reconstituted medium.

Bacto proteose peptone #3	10.0	g
Bacto yeast extract	10.0	g
Sodium chloride (reagent grade)	5.0	g
Sodium glycerphosphate	10.0	g
Maltose (CP)	20.0	g
Lactose (CP)	1.0	g
Sodium azide (Eastman)	0.4	g
Sodium carbonate (Na_2CO_3 reagent grade)	0.636	g
Brom cresol purple (water soluble)	0.015	g
Bacto agar	20.0	g

b Reagent

2, 3, 5-Triphenyl tetrazolium chloride reagent (TPTC)

This reagent is prepared by making a 1% aqueous solution of the above chemical passing it through a Seitz filter or membrane filter. It can be kept in the refrigerator in a screw-capped tube until used.

c The dehydrated medium described above is prepared for laboratory use as follows:

Suspend 7.64 grams of the dehydrated medium in 100 ml of distilled water in a flask with an aluminum foil cover.

Place the flask in a boiling waterbath, melt the dehydrated medium, and leave in the boiling waterbath an addional 5 minutes.

Cool the medium to 50°-60° C, add 1.0 ml of the TPTC reagent, and mix.

For membrane filter studies, pour 5-8 ml in each 50 mm glass or plastic culture dish or enough to make a layer approximately 1/8" thick. Be sure to pour plates before agar cools and solidifies.

For plate counts, pour as for standard agar plate counts.

NOTE: Plastic dishes containing media may be stored in a dark, cool place up to 30 days without change in productivity of the medium, provided that no dehydration occurs. Plastic dishes may be incubated in an ordinary air incubator. Glass dishes must be incubated in an atmosphere with saturated humidity.

2 Apparatus, and materials as given in Table 1.

3 General procedure is as given in I.

Special instructions

a Incubate 48 hours, inverted with 100% relative humidity after filtration.

b After incubation, remove the cultures from the incubator, and count colonies under wide field binocular dissecting microscope, with magnification set at 10X or 20X. Fecal streptococcus colonies are pale pink to dark wine-color. In size they range from barely visible to approximately 2mm in diameter. Colorless colonies are not counted.

c Report fecal streptococcus count per 100 ml of sample. This is computed as follows:

No. fecal streptococci per 100 ml =

$$\frac{\text{No. fecal streptococcus colonies}}{\text{Sample filtration volume in ml}} \times 100$$

B Verification of Streptococcus Colonies

1 Verification of colony identification may be required in waters containing large numbers of Micrococcus organisms. This has been noted particularly with bathing waters, but the problem is by no means limited to such waters.

2 A verification procedure is described in "Standard Methods for the Examination of Water and Wastewater," 14th ed, (1975). The worker should use this reference for the step-by-step procedure.

IV PROCEDURES FOR USE OF MEMBRANE FILTER FIELD UNITS

A Culture Media

1 The standard coliform media used with laboratory tests are used.

2 To simplify field operations, it is suggested that the medium be sent to the field, preweighed, in vials or capped culture tubes. The medium then requires only the addition of a suitable volume of distilled water-ethanol prior to sterilization.

3 Sterilization procedures in the field are the same as for laboratory methods.

4 Laboratory preparation of the media, ready for use, would be permissible provided that the required limitations on time and conditions of storage are met.

B Operation of Millipore Water Testing Kit, Bacteriological

1 Supporting supplies and equipment are the same as for the laboratory procedures.

2 Set the incubator voltage selector switch to the voltage of the available supply, turn on the unit and adjust as necessary to establish operating incubator temperature at 35 ± 0.5°C.

3 Sterilize the funnel unit assembly by exposure to formaldehyde or by immersion in boiling water. If a laboratory autoclave is available, this is preferred.

Formaldehyde is produced by soaking an asbestos ring (in the funnel base) with methanol, igniting, and after a few seconds of burning, closing the unit by placing the stainless steel flask over the funnel and base. This results in incomplete combustion of the methanol, whereby formaldehyde is produced. Leave the unit closed for 15 minutes to allow adequate exposure to formaldehyde.

4 Filtration and incubation procedures correspond with laboratory methods.

5 The unit is supplied with a booklet containing detailed step-by-step operational procedures. The worker using the equipment should become completely versed in its contents and application.

C Other commercially available field kits should be used according to manufacturer's instructions. It is emphasized that the required standards of performance are manditory for field devices as for laboratory equipment.

D Counting of Colonies on Membrane Filters

1 Equipment and materials

Membrane filter cultures to be examined

Illumination source

Simple lens, 2X to 6X magnification

Hand tally (optional)

2 Procedure

a Remove the cultures from the incubator and arrange them in numerical sequence.

b Set up illumination source as that light will originate from an area perpendicular to the plane of membrane filters being examined. A small fluorescent lamp is ideal for the purpose. It is highly desirable that a simple lens be attached to the light source.

c Examine results. Count all coliform and noncoliform colonies. Coliform and noncoliform colonies. Coliform colonies have a "metallic" surface sheen under reflected light, which may cover the entire colony or may appear only on the center. Noncoliform colonies range from colorless to pink or red, but do not have the characteristic "metallic" sheen.

d Enter the colony counts in the data sheets.

e Enter the coliform count per 100 ml of sample for each membrane having a countable number of coliform colonies. Computation is as follows:

No. coliform per 100 ml =

$$\frac{\text{No. coliform colonies on MF}}{\text{No. milliliters sample filtered}} \times 100$$

REFERENCES

1 Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WPCF 14th Edition. 1975.

2 McCarthy, J.A., Delaney, J.E. and Grasso, R.J. Measuring Coliforms in Water. Water and Sewage Works. 1961: R-426-31. 1961.

This outline was prepared by H. L. Jeter, former Chief, Program Support Training branch, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268

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